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PANTOTHENIC ACID AND THE NODULE BACTERIA-LEGUME SYMBIOSIS

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The elementary facts regarding the symbiotic relationship between leguminous plants and the rhizobia have been known for many years but the chemical basis upon which this symbiosis rests is as yet imperfectly understood. It is not known with certainty what chemical entities pass from the bacteria to the host and vice versa. The net result of the partnership is nitrogen fixation, and heretofore it has been considered that the primary reaction or series of reactions involving elemental nitrogen are carried on by the bacteria. Fixation has not been demonstrated, however, except in the symbiotic relationship. It is not known definitely whether the fixation of nitrogen should be ascribed to the immediate activities of the bacteria living in the nodules, or to the activities of the host plant resulting from the bacterial infection. Activities of rhizobia other than utilization of atmospheric nitrogen have previously not been considered in explaining the direct benefit of symbiosis to the leguminous plant.

The nutritional need of *Rhizobium meliloti* for an unknown substance which they designate as "coenzyme R" has recently been demonstrated by Allison, Hoover and Burk.[1] Their results on this have been confirmed in our laboratory; moreover, it was found that alfalfa is a very rich source of this unknown substance, which we designate a "nutrilite."[2] One of the chemical entities passing from the alfalfa plant to the rhizobia is thus indicated to be this as yet poorly characterized nutrilite which is necessary for growth of the bacteria.

That one of the significant chemical substances which passes from the bacteria to the host plant is panthothenic acid, and that this substance has a profound effect on the growth of the alfalfa and on carbohydrate anabolism but does not (alone) enable the alfalfa plant to fix atmospheric nitrogen, is indicated by the experimental work herewith presented.

Pantothenic acid[3] has been found in every organic tissue examined whether of animal, bacterial or plant origin. Rhizobia are no exception; in the case of this organism as with certain other bacteria and molds, pantothenic acid is produced as the organism grows. A series of 25 ml. cultures of *Rhizobium meliloti* were grown in a synthetic medium[4] containing ordinary sugar, which carries a small amount of the rhizobium nutrilite. At the end of each successive day a culture was sterilized by heat, the medium filtered clear, using kieselguhr, and its pantothenic acid content determined by its effect on yeast growth under controlled conditions.[5] The original culture medium was practically free from pantothenic acid but after successive days' growth the amount present expressed in arbitrary units was 0.39, 0.78, 1.63, 5.46, respectively. It is therefore evident that the rhizobia are capable of producing pantothenic acid; in the nodule this could be passed on to the alfalfa plant.

Other work from this laboratory (to be published in *Plant Physiology*) has indicated that green plants may be stimulated in their growth by pantothenic acid. The following experiment was performed to check the effect of a pantothenic acid concentrate upon the growth of alfalfa, and to determine whether uninoculated alfalfa seedlings, when furnished with pantothenic acid, are able to fix atmospheric nitrogen.

Alfalfa was grown in three quadruplicate sets in Pyrex test tubes ($2^1/_2'' \times 24''$). Five hundred grams of quartz sand and 150 ml. of modified Crone's[6] solution (nitrogen free) were placed in each tube; these were capped with gauze-enclosed cotton and autoclaved 6 hours at 15 lb. pressure. Four of the tubes served as a control, four were inoculated with *Rhizobium meliloti*, and to each of the other four was added 2 micrograms (6 units) only of a pantothenic acid concentrate in the form of a calcium salt. Each of the twelve tubes was seeded under aseptic conditions with 6–8 alfalfa seeds which had been sterilized with mercuric chloride, and the cultures were placed in the greenhouse. After four days it was apparent that the plants in the tubes with the minute amount of pantothenic acid were uniformly larger than either of the other sets. At the end of two weeks the difference between the pantothenic acid treated plants and the controls was striking, while the inoculated seedlings were almost, but not quite, as vigorous as those which were treated with pantothenic acid and kept sterile. At this time an additional quantity (10 micrograms) of pantothenic acid was added to each of the tubes which had been treated originally. Subsequent results make it doubtful whether this second addition had any beneficial effect. After one month the growth was very slow, perhaps due to the short winter days, and the pantothenic treated plants were a shade lighter green than the inoculated plants. The seedlings were then harvested, measured weighed (air dry) and the nitrogen determined by a micro-Kjeldahl method,[7] with the following results:

TABLE 1

EFFECT OF PANTOTHENIC ACID ON ALFALFA

	TUBE NO.	NUMBER OF PLANTS	AVERAGE LENGTH OF STEMS (MM.)	TOTAL WT. OF ROOTS (MG.)	TOTAL WT. OF STEMS (MG.)	TOTAL WT. OF WHOLE PLANTS (MG.)	TOTAL N OF WHOLE PLANTS (MG.)	N/PLANT (MG.)
Controls	1	6	30	9.5	19.5	29.0	0.882	0.146
	2	6	34	12.5	14.5	27.0	0.750	0.125
	3	8	39	14.5	27.5	42.0	1.382	0.173
	4	5	20	7	12.5	19.5	0.790	0.158
Inoculated	5	4	50	7	22.5	39.5	0.832	0.208
	6	6	54	12	31.0	43.0	1.304	0.218
	7	5	36	8.5	19.5	28.0	0.864	0.173
	8	4	41	8.5	16.0	24.5	0.722	0.180
Pantothenic acid treated	9	6	50	19	35.0	54.0	1.030	0.172
	10	6	55	20	35.5	55.5	1.030	0.172
	11	6	53	13	33.5	47.0	1.076	0.178
	12	6	47	10	27.0	37.0	0.930	0.152

TABLE 2

GRAND AVERAGE OF VALUES FROM TABLE 1

	LENGTH/PLANT	WT./PLANT	TOTAL N/PLANT	% N/PLANT
Controls	31 mm.	4.70 mg.	0.152 mg.	3.23%
Inoculated	45 mm.	7.10 mg.	0.196 mg.	2.76%
Pantothenic acid	51 mm.	8.05 mg.	0.169 mg.	2.10%

Heretofore the favorable effects resulting from inoculating alfalfa and other legumes have been explained solely on the theory that the organisms fix nitrogen. On the basis of our results this position is no longer tenable. Perusal of tables 1 and 2 shows that minute amounts of pantothenic acid exert a striking effect on alfalfa in the early, and often critical, stage of growth. Since it has been shown that *Rhizobium meliloti* produces significant amounts of pantothenic acid, it is therefore likely that a considerable part of the stimulatory effect of the bacteria is due to pantothenic acid synthesized and excreted by the organism in the nodule and passed on to the plant.

From the fact that the weight of the pantothenic acid treated plants is greater than that of either the controls or the inoculated plants, and from the lack of a significant increase in nitrogen over the controls, it is evident that pantothenic acid alone is not the deciding factor in the nitrogen fixation process, but rather that it plays an important part in the carbohydrate anabolism of the plant. This is in keeping with the fact that it has been shown in this laboratory to exert a powerful effect on both the aerobic and anaerobic respiration of yeast. Further experiments are now in progress.

[1] Allison, Hoover and Burke, *Science*, **78**, 217 (1933).

[2] Williams, *Ibid.*, **67**, 607 (1928).

[3] Williams, Lyman, Goodyear, Truesdail and Holaday, *Jour. Am. Chem. Soc*, **55**, 2912 (1933).

[4] Allison and Hoover, *Jour. Bact.*, **27**, 561 (1934).

[5] Williams, McAlister and Roehm, *Jour. Biol. Chem.*, **38**, 315 (1929).

[6] Fred, Baldwin and McCoy, *Univ. of Wis. Studies in Science*, No. 5, "Root Nodule Bacteria and Leguminous Plants," **266** (1932).

[7] Koch and McMeekin, *Jour. Am. Chem. Soc.*, **46**, 2066 (1924).

LABILITY OF THE BASAL METABOLISM OF THE DAIRY COW

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Approximately fifty years ago Rubner emphasized the concept that all warm-blooded animals give off heat in direct proportion to their surface areas and at an essentially constant rate, irrespective of difference in species, of one thousand calories per square meter of body surface per 24 hours. This theory has stimulated a great deal of research and has been of invaluable service in that it has led to the accumulation of much information with regard to the heat production of various animals. Most writers even at the present day believe in the general applicability of this surface-area law, in spite of the fact that increasing evidence is appearing to show that the heat production per unit of body surface is by no means so uniform with all warm-blooded animals as was originally thought. The production of heat in the animal body is the sum of the endogenous heat production necessary for the maintenance of minimum vital activity and the increases in heat production caused by superimposed factors, primarily the absorption of food and muscular activity. By studying humans and animals in complete muscular repose, after the digestive processes have ceased, measurements can be made that supposedly represent the true basal metabolism and hence are comparable among different animal species. It was soon noted, however, that even within any given species factors other than digestion and muscular activity play an appreciable rôle in the heat production, particularly age, and specific differences due to weight, height and sex have been found.

Study of the basal metabolism of any given species of animal has two specific values. It contributes to the knowledge of the basal metabolism of that given species and the variability existing within the species and

enables comparison with the metabolism of other species. It also furnishes a baseline for measuring the effect of any superimposed factor, such as digestion or muscular work, within the species itself. In the feeding of domestic animals knowledge of the effect upon metabolism of the work of digestion is of practical, economic importance. The multiplicity of feeds for domestic animals, represented by roughages such as hay and concentrates such as cornmeal, oats, bran and wheat, and the great differences in the market values of these feeds have led to a survey of their digestibility. Careful study has shown that the digestibility is highest with the concentrates and lowest with the roughages. But more important and less well known is the tremendous stimulus to metabolic activity as the result of the ingestion of any food. This stimulus of the food is determined by comparison of the metabolism during the active processes of digestion with the metabolism when food is no longer being absorbed from the intestinal tract. With man the post-absorptive condition is reached after a fairly short period of fasting, usually within 12 or 14 hours, but with large ruminants a much longer period of fasting is necessary.

Belief in the constancy of basal metabolism has led most workers to accept too readily a few experiments or, indeed, only one experiment to establish this important baseline prior to or following a series of experiments in which the effects of various kinds or amounts of feed are studied. In a coöperative investigation between the Nutrition Laboratory of the Carnegie Institution of Washington and the Laboratory for Animal Nutrition at the New Hampshire Agricultural Experiment Station we have been occupied for many years in studying the metabolism of large ruminants, namely, cows and steers and, in addition, the horse. The effects of the ingestion of the various roughages and concentrates upon the heat production of these animals are most striking, relatively enormous increases in the basal metabolism having been regularly noted. In these investigations we did not accept one basal determination for comparison with a series of feed experiments, but considered that the most logical method was to repeat the measurement of the basal metabolism of each animal each time that the stimulating effect of a given feed was to be studied. Our procedure was therefore as follows:

The animal was fed for at least four weeks on a constant ration, maintenance or somewhat better than maintenance, at the end of which time the metabolism for two consecutive 24-hour periods was measured with the animal (while still on feed) inside a respiration chamber. Feed was then withdrawn, and the metabolism was again measured on the fourth and fifth days of fasting, at which time essentially a plateau in the metabolism had been reached, the respiratory quotients approached that of fat, and the methane production was very low.

Five Holstein (ca. 600 kg.) and four Jersey (ca. 300 kg.) cows, ranging

in age from 3 to 15 years, were studied in this way. In most instances they were dry, in a few cases pregnant or lactating. The experimental series with any one animal extended from two months to three and one-half years. The feeds given were roughages alone, concentrates alone or green grass (pasture). The roughages included six different hays, of varying protein content and of early and late cuttings. The concentrates were either cornmeal alone or linseed meal supplemented by wheat bran. At no time were the cows undernourished. During both the digestion and the fasting period they were kept at a constant, controlled environmental temperature of about 20°C.

In accordance with Rubner's concept, the heat production of the non-digesting, muscularly inactive animal should be constant per square meter of body surface per 24 hours. But with the cows that we studied a wholly unsuspected lability was found in the basal metabolism of one and the same animal, a lability of an order of magnitude altogether too great to be attributed to any possible slight technical error. Furthermore the accuracy of the respiration apparatus was frequently controlled by the introduction of a known, weighed amount of carbon-dioxide gas, which was subsequently completely recovered by the aliquoting, chemical and metering methods employed. The extremes noted in our basal metabolism measurements on dairy cows are reported in table 1, the heat values being expressed as calories per 500 kilograms of body weight per 24 hours for the animal in the lying position. Similar variability is seen if the calculations are based on the surface area.

TABLE 1

COW	EXTREMES IN BASAL METABOLISM	PER CENT RANGE
H-I	5690– 6260	10
H-IV	4820– 8190	70
H-V	4500– 8510	90
H-VI	7150– 9000	25
J-I	7310– 9830	35
J-II	5710–10750	90
J-IV	5450– 8890	65

In the experiments in which these extremes were noted the cows were non-lactating and were not pregnant, and the changes in body weight of any given animal were insignificant. In an analysis of the conditions under which these variations in metabolism occurred one outstanding fact appeared in every instance, that is, that when the cows were studied during the pasture season, for example, in June, the basal metabolism (4th–5th day fasting) was at a much higher level than at any other time of the year. There was, furthermore, a tendency for the lowest values to appear with many of the animals during the month of March.

Another striking fact is that there occurred, too frequently to be dis-

regarded, a profound change in the metabolic level within a period of two months, during which time it is inconceivable that the nutritive state of the animal had been seriously altered by the substitution of one roughage for another or of a concentrate or grass for roughage. The considerable percentage differences in the metabolic levels (calories per 500 kg. per 24 hours, lying) occurring within such a short time are shown in table 2.

TABLE 2

COW	DATE	BASAL METABOLISM	PER CENT RANGE
H-IV	Sept., 1934	7700	50
	Nov., 1934	5140	30
	Jan., 1935	6790	
H-V	Dec., 1932	4890	35
	Jan., 1933	6690	
	Mar., 1934	5900	45
	May, 1934	8510	
	Oct., 1934	8310	85
	Nov., 1934	4500	60
	Dec., 1934	7210	
J-II	Dec., 1934	5710	40
	Jan., 1935	8090	
J-IV	Jan., 1935	8890	65
	Mar., 1935	5450	

The body weights in these instances had not undergone any significant change, and although the feeds given were not the same in any pair of experiments, it must be remembered that all these measurements were made on the fourth and fifth days of fasting, under which conditions the immediate effects of the previous ingestion of food are ruled out, absorption has ceased, and the measurement represents only the metabolic activity of the cells of the animal body.

The important fact brought out by these experiments is that the basal metabolism of the dairy cow is by no means constant, even for a relatively short time, and it would be extremely difficult to give one representative basal value for dairy cows in general. This lability in the basal metabolism of the dairy cow is, upon analysis, not surprising, when one considers that the cow is an animal whose diet, both qualitatively and quantitatively, has been selected by man for the express purpose of transforming into milk as much as possible of material (hays and grass) otherwise inedible by man. The selection by breeding of cows that show great productivity in milk has resulted in the development of an animal perhaps paralleled nowhere else in the entire zoölogical kingdom. This selective breeding has been accompanied by special, forced feeding and artificial conditions, which doubtless have contributed not a little to this great flexibility in metabolism. From the practical standpoint it is clearly demon-

strated that any experiments on cows designed to study the specific dynamic action or the stimulating effect of food must be so planned that the baseline is determined as near as possible to the time of the measurement of the metabolism when the animal is fully stimulated by the feed. Thus it is inconceivable that for a series of feed experiments carried out throughout the winter one basal value determined either at the beginning or the end of the series will be applicable to the various feed measurements.

Although our particular findings here reported apply to the dairy cow, an animal whose productive activity has been perhaps most pronouncedly governed by man by feeding and selective breeding, a lability in metabolism (not so marked) has also been noted by us with the sheep and the horse. These observations have a practical, economic value in assessing the nutritive values of different feeds, but what is more important from the physiological standpoint, they also emphasize the possibility of a tremendous variability in the endogenous metabolism of any adult organism, even when there are no appreciable changes in body weight.

(The details of this investigation on the variability in the basal metabolism of large domestic animals and a critical analysis of the results will appear shortly in a publication from the University of New Hampshire.)

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*THE PROVINCETOWN, MASSACHUSETTS, EARTHQUAKE OF APRIL 23, 1935, AND DATA FOR INVESTIGATING NEW ENGLAND'S SEISMICITY**

By L. D. LEET**

HARVARD UNIVERSITY SEISMOGRAPH STATION

Communicated May 10, 1935

Provincetown, Massachusetts, and the tip of Cape Cod were shaken by an earthquake of very slight intensity, accompanied by earthquake sounds, on the evening of April 23, 1935 (local time). The shock was well recorded by the Benioff short-period seismographs at the Oak Ridge Observatory of the Harvard University Seismograph Station, Harvard, Massachusetts. Mr. Gibbs and the writer visited the Cape the following day to obtain reports from local residents, particularly men on watch at the many Coast Guard stations.

In figure 1 the shaded portion of Cape Cod represents the area over which the earthquake was felt or heard. Strongest shaking was reported by R. J. Davey, on watch at the Race Point Coast Guard tower. Dr. P. J. Eaton, of Provincetown, described the sound as "a loud report,

followed by a couple of reverberations, such as might have been made by a big gun fired at sea, or a not too distant single peal of thunder." Some Provincetown residents did not recognize the vibrations as anything more unusual than the passing of a heavy truck. The Cahoons Hollow Coast Guardsman on beach duty reported hearing the rumble, but felt no vibration. The Pamet River man neither heard nor felt anything unusual, nor did men at Nauset and other stations on the Cape, as well as Manomet Point, near Plymouth, on the mainland.

Figure 2 shows a copy of the Harvard records. The first longitudinal wave, designated by P in the figure, arrived as a rarefaction at 01–24–25.5 Greenwich Civil Time, April 24th. 01 hour GCT was 8:00 P. M. Eastern Standard Time, April 23rd. The first transverse wave, S, was recorded at

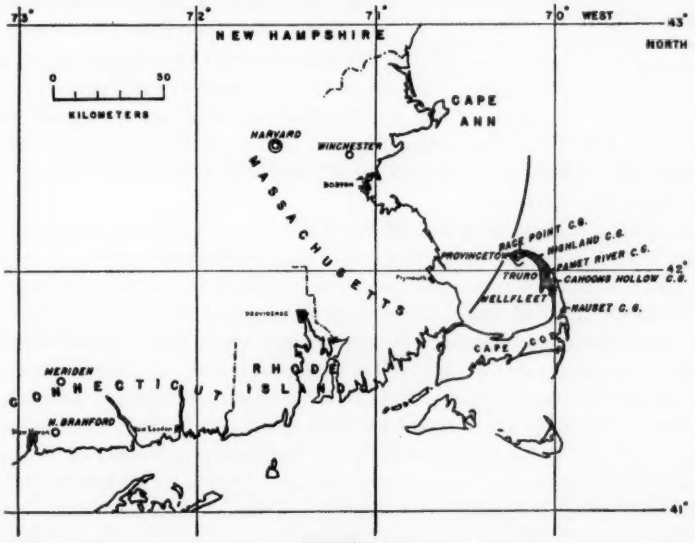

FIGURE 1

01–24–39.5. No North-South component record is available, because both horizontal Benioffs were oriented East-West at the time, for test purposes.

The time-interval between P and S at a recording station, in this case, 14.0 seconds, is a function of the distance to their source. The form of this function has to be determined empirically, and the Harvard Seismograph Station is assembling data for the first determination for New England. Existing tables for the travel-times of P and S, and the S-P interval as a function of distance, which are used for analyzing records from distant earthquakes, are known to be no better than poor approximations at distances less than 500 kilometers.

Large dynamite blasts used in certain types of routine quarrying can supply the necessary data. Through the willing and extremely essential coöperation of the Atlas, Du Pont and Hercules powder companies, as well as the managements of the various quarries, advance notice of large blasts reaches the Station. Steps are then taken to determine the exact instant of firing. The resulting vibrations recorded on the seismographs, accordingly, have started at a known time and traveled a known distance. The Harvard Station has undertaken an extended program of such blast registration. The objectives of this research include knowledge concerning the surface-layers of the earth in New England, as well as statistical data of several kinds on local earthquakes. Some of the first results, however, are directly applicable to the interpretation of such records as those of the Provincetown earthquake.

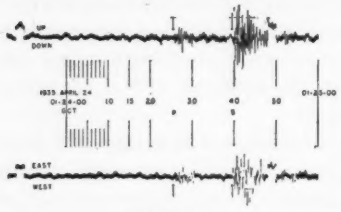

FIGURE 2

Records of Provincetown earthquake of April 24, 1935, obtained on Benioff short-period seismographs at Harvard, Massachusetts. $\Delta = 117.5$ km. $H = 01\text{-}24\text{-}06.7$ GCT.

In figure 3 are copies of Benioff vertical-component records of the vibrations from three timed quarry blasts. The Winchester record is a representative sample of nine obtained through the coöperation of officials of the General Crushed Stone Co., quarry operators, and the Boston office of the Atlas Powder Company. It was timed by Mr. Gibbs and the writer, using a portable short-wave wireless receiver picking up continuous time-signals on 7350 kc. from the Dominion Observatory, Ottawa, Canada.

The Meriden blast was timed by Professor L. B. Slichter of Massachusetts Institute of Technology, using an oscillographic record of Western Union time-signals and the instant-of-blast. Operators of the York Hill Quarry, and Mr. J. Barab, of the Hercules Powder Company, coöperated.

The North Branford blast was similarly timed by Professor Slichter through the coöperation of the quarry owners and Mr. S. R. Russell of the Du Pont Company.

The exact location of each blast was determined with high precision by Mr. Weld Arnold, of the Harvard Institute of Geographical Exploration, using a combination of aerial photographs and astronomical position observations.

From these data, the graph in figure 4 was plotted, showing travel-times for P and S at the distances involved, together with the S-P interval. On this graph, it is evident that an S-P interval of 14.0 seconds, found for the Provincetown earthquake, corresponds to a distance of 117.5 kilometers (73 miles). The travel-time for P at that distance is shown as 18.8 seconds,

so the computed time of occurrence for the earthquake becomes 01–24–06.7.

An arc of the circle whose radius is 117.5 kilometers, about Harvard, Massachusetts, as a center, is shown in figure 1. If we assume that the focus, or source of the earthquake vibrations, was near or at the surface of the earth, as it must have been if the shaking of so small a shock was felt at all, it was located on that circle. Taking into account the reports of rapidly diminishing perceptibility in all directions on the Cape from the Race Point Coast Guard tower, the approximate position of the focus is adopted as 42° 10′ North Latitude, 70° 13′ West Longitude, the southerly end of Stellwagen Bank.

The only unusual feature of the Provincetown earthquake, among the past year's records of local earthquakes at Harvard, is the accident of location where reports of felt motion, combined with instrumental data

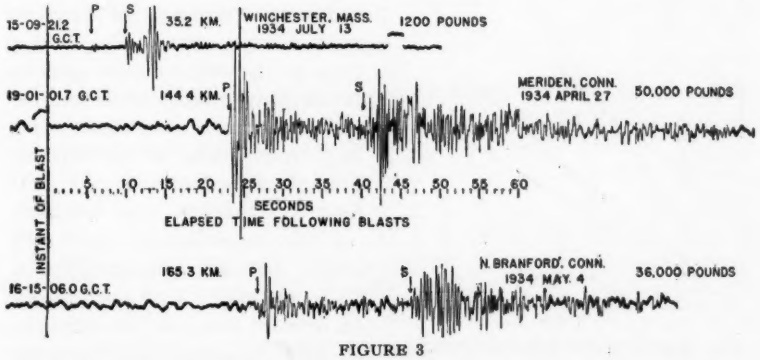

FIGURE 3

Vibrations from blasts as recorded on Benioff short-period vertical-component seismograph at Harvard, Masschusetts.

from a single seismograph station, warrant an approximate determination of position of the focus. The Benioff vertical-component seismograph recorded 64 local shocks between March 31, 1934, and February 1, 1935 with phases sharp enough to permit *S-P* distance computations. One of these, April 15, 1934, was reported felt over a wide area in Vermont. During August 2, 1934, several reports of felt tremors were received from places along the Maine, New Hampshire and Massachusetts coast. There were no reports connected with the others, of which 32 were between 100 and 150 kilometers from Harvard.

On the basis of the performance of this vertical-component Benioff, horizontal-components with identical operating constants were installed at the Harvard Station on April 1, 1935. In favorable cases, these will permit computation of approximate azimuths and angles of emergence,

two of many important results of having records of the complete ground motion.

The low ratio of felt to recorded shocks during this first year of observation with a Benioff seismograph, which, in a group including Milne-Shaws and Wood-Andersons, is the only one to give usable, or any, records of earthquakes as small as most of these, is a strong reminder that reports of felt shocks during the past 300 years probably offer a very incomplete, if not totally useless, basis for accurate delineations of regions of active faulting in New England. The fact that only one case out of sixty-five, that of Provincetown, provided both instrumental and field reports which could be combined to obtain a reasonably fair estimate of location, illustrates strikingly the need for more seismographs of the Benioff type in the area to make possible instrumental triangulation and frequent, accurate locations. The blast-record data of the Harvard Station are accumulating so as to furnish ultimately the necessary reference materials, but more sufficiently sensitive seismographic installations are vital to any program for detailed study of the seismicity of New England. The potential services of a proper network of stations, to engineers and communities in general, as well as to science, need no emphasis. The possibilities of coördinated regional investigations are shown by results already obtained in California.

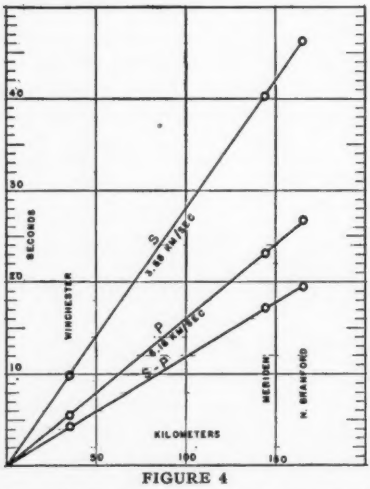

FIGURE 4

Time-distance graph based on records of figure 3.

Summary.—A very slight earthquake was felt along the tip of Cape Cod, Massachusetts, on the evening of April 23, 1935 (local time). Instrumental records at Harvard, Massachusetts, combined with reports from the shaken area, lead to the following conclusions:

(1) Position of the focus approximately:

42° 10′ North Latitude
70° 13′ West Longitude

(2) Time at focus:

01–24–06.7 Greenwich Civil Time, April 24, 1935
(8–24–06.7 P. M. Eastern Standard Time, April 23, 1935)

Some data are given from the early results of the Harvard Seismograph Station's program of recording dynamite blasts, which give travel-times for P and S for use in computing local earthquake distances. A low ratio of felt to recorded earthquakes, and the urgent need for more Benioff installations in the area, are cited.

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** Seismologist in Charge.

EQUATORIAL LONGITUDE EFFECTS ON COSMIC RAYS

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At the meeting of the National Academy just a year ago Dr. Neher and I presented our evidence for a previously unsuspected effect in cosmic rays which we termed the "longitude effect." This effect had been brought to light by the sending of vibration-free self-recording electroscopes on a number of voyages from Los Angeles to Mollendo, Peru, and back, and at the same time on a Dollar Line trip around the world. The very accurate records obtained on these voyages, when studied carefully in the laboratory in January 1934, brought to light the fact that in going from the north temperate zone into the equatorial belt the decrease in the sea level cosmic ray intensity is only 8 per cent between San Francisco and Mollendo while it is 12 per cent when the passage from the temperate zone into the equatorial belt takes place on the other side of the world, that is, in the region of Singapore and Batavia. Several months later we learned that Professor J. Clay, of Amsterdam, had observed a similar qualitative difference in two voyages which he made at essentially the same time as those made by us, one of his voyages taking him between Amsterdam and Batavia around the Cape of Good Hope and the other through the Suez Canal. Professor Clay published a paper in the March number of the Dutch magazine *Physica*, in which he referred very briefly to these results, so that priority in the publication of the discovery of the longitude effect belongs to Professor Clay. The agreement however in the results found simultaneously and independently in different parts of the earth by different observers puts beyond doubt the reality of the effect.

The importance of the effect is obvious when it is reflected that the charged particles which alone can be responsible for this longitude effect must have gained their deflections in the earth's magnetic field at dis-

tances thousands of miles above the earth's surface, so that the existence of the effect means that, contrary to the well-known gaussian assumption, the earth's magnetic field even at distances thousands of miles out in space is essentially dissymmetrical with respect to any line passing through the center of the earth. The longitude effect in cosmic rays provides then the first means of making a survey of the extent and nature of that dissymmetry. All the sea level surveys of the earth's magnetic field which have been made in such numbers during the past three hundred years and which have had such utility for the navigator have been limited to the measurement of variations in the components of the magnetic field at *the surface of the earth.*

In view of the significance of such a survey, Dr. Neher and I have gone intensively at the problem of getting accurate data as fast as possible on the sea level cosmic ray intensities. We have sent one shielded electroscope from Los Angeles to Sydney on the Matson Line, and also an unshielded electroscope over the same route. We have sent another electroscope to Peru, which was placed by our collaborator, Dr. Korff, on a freighter which made the trip from Mollendo through the Canal to Liverpool and then from Liverpool back around South America through the Straits of Magellan, and then up the west coast of South America to Valparaiso and Mollendo. We have also sent a second electroscope on a Dollar Line trip around the world. In addition we now have other instruments which are traversing the Southern Hemisphere on the Franconia of the Cunard Line.

The results of all the voyages thus far completed are in agreement with the general conclusions announced a year ago, namely, that the dip in the cosmic ray intensities in crossing the equatorial belt along the west coast of South America is 8 per cent; that in crossing this belt farther west in the Pacific in the route from Los Angeles to Sydney this equatorial dip is 10 per cent; in crossing in the neighborhood of Singapore and Batavia it is 12 per cent; in crossing through the Atlantic from Liverpool to the Straits of Magellan it is $8^1/_2$ per cent. Within the limits of our experimental uncertainty which does not exceed one per cent, these figures are the same whether we use electroscopes shielded with ten centimeters of lead or whether we use unshielded electroscopes.

Another important result is that in the neighborhood of Los Angeles the equatorial dip in intensity sets in with a good deal of accuracy at a magnetic latitude of 41°, that is, just a few miles south of Pasadena. Similarly, in the Atlantic Ocean the equatorial dip sets in apparently quite sharply very close to 41° or 42° magnetic latitude.

Another result of the sea level surveys is that there seems to be very nearly symmetry between the effects observed in the Northern Hemisphere and in the Southern. The results are not complete enough to make this

symmetry a certainty. This report is rather one of progress, for the sea level investigation is still under way.

A careful study of the longitude effect in the equatorial belt *at high altitudes* is even more important than at sea level for out of it must come pretty definite and conclusive evidence of the existence or non-existence of photonic radiations coming in from outside and producing in part the ionizing effects actually observed in our atmosphere. Dr. Neher and I are therefore now in the process of making in collaboration with Dr. S. A. Korff high altitude flights with accurate recording electroscopes first in Peru and second in the Asiatic area represented by the Philippines, Java and Singapore. We can present as yet only the results obtained in Peru up to 26,100 ft. These show a continuous exponential rise of the ionization with a coefficient 0.5 per meter of water without any tendency as yet to be influenced by the linear rate of increase with altitude which we have estimated should be obtained if there were any large charged-particle component as distinct from photonic component of the radiations in the equatorial belt. Our former conclusion as to this exponential rise in intensity up to and considerably beyond our former measured levels is thus nicely confirmed. Within a month or so we hope to have similar records from the Philippines. If these should show very markedly lower ionization at high altitude on that side of the earth, such as Clay has recently reported in preliminary and admittedly inaccurate work, there will be no possibility in our judgment of explaining this difference on the theory of incoming photons, so that we hope to have very soon a rather definite answer to this important question through our study of the high altitude longitude effect in the equatorial belt.

These two surveys, one at sea level and one at high altitudes, are being supported by grants from the Carnegie Corporation of New York administered by the Carnegie Institution of Washington, to which institutions we desire to express our thanks for this assistance.

SPHEROIDAL FUNCTIONS OF THE SECOND KIND

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In a recent note in these PROCEEDINGS,[1] henceforth to be referred to as I, certain solutions of the equation

$$(1 - z^2)w'' - 2(a + 1)zw' + (b - c^2z^2)w = 0 \qquad (1)$$

were defined. Corresponding to a discrete set of characteristic values of the parameter b_l there exist solutions of the type

$$Re^1_{a,l}(c, z) = (cz)^{-a-1/2} \sum_n{}' a^l_n J_{n+a+1/2}(cz), \qquad (I\text{-}10)$$

convergent throughout the entire z-plane and behaving as $(cz)^{-a-1}$ sin $\left(cz - \frac{l+a}{2}\pi\right)$ as $z \longrightarrow \infty$. In the neighborhood of $z = 0$ a formal solution may be obtained in terms of an expansion in hypergeometric functions,

$$Se^1_{a,l}(c, z) = \sum_n{}' d^l_n T^a_n(z), \qquad (I\text{-}8)$$

which for the characteristic values b_l is also bounded at the singularities $z = \pm 1$. With the aid of the integral representation (I-18) it was shown that (I-8) and (I-10) are proportional,

$$Se^1_{a,l}(c, z) = \lambda_l k_l \, Re^1_{a,l}(c, z), \qquad (I\text{-}26)$$

and that the expansion coefficients are related by the simple expression (I-22).

An independent particular solution of (1) behaving at infinity as $(cz)^{-a-1}$ cos $\left(cz - \frac{l+a}{2}\pi\right)$ may be defined formally by

$$R^2_{a,l}(c, z) = (cz)^{-a-1/2} \sum_n{}' a^l_n N_{n+a+1/2}(cz). \qquad (I\text{-}11)$$

This expansion, however, diverges in general at the branch points $z = \pm 1$ and consequently is usable only for large values of the independent variable. It is the object of the present note to define a second solution valid in the neighborhood of $z = 0$, and to determine its analytic connection with the functions (I-10), (I-11).

By analogy with the definition (I-4) of the functions T^a_n, let

$$\mathbf{T}^a_n(z) = (1 - z^2)^{-a/2} Q^a_{n+a}(z), \qquad (2)$$

where the Q^a_{n+a} are Associated Legendre functions of the second kind as defined by Hobson.[2] If $R(n + a + 1) > 0$,

$$\mathbf{T}^a_n = \frac{e^{a\pi i}\,\Gamma(n + 2a + 1)}{2^{n+a+1}\,\Gamma(n + a + 1)} \int_{-1}^{1} (1 - t^2)^{n+a}\,(z - t)^{-n-2a-1}\,dt. \quad (3)$$

Since both the T^a_n and the $\mathbf{T}^a_n$ thus defined satisfy the same recursion formulas, a second formal solution of (1) may be defined,

$$Se^2_{a,l}(c, z) = \sum_n{}' d^l_n \mathbf{T}^a_n(z), \quad (4)$$

which may be expected to converge in the neighborhood of $z = 0$, since $\sum_n{}' d^l_n$ is bounded when b assumes one of the characteristic values b_l.

It is known that (1) is satisfied by the Laplace transform

$$w(z) = \int_C e^{icst}(1 - t^2)^a\,u(t)dt, \quad (I\text{-}18)$$

where $u(t)$ itself satisfies (1) and the contour C is to be determined by the bilinear concomitant

$$\left| e^{icst}\,(1 - t^2)^{a+1}\left[icszu - \frac{du}{dt}\right]\right|_C \equiv 0. \quad (I\text{-}19)$$

As in I let $u(t) = Se^1_{a,l}(t) = \lambda_l k_l Re^1_{a,l}(ct)$. If $t \longrightarrow \infty$ and $|arg\ ct| < \pi$,

$$Se^1_{l,a}\,(t) \longrightarrow \lambda_l k_l\,(ct)^{-a-1} \sin\left(ct - \frac{l + a}{2}\pi\right).$$

Let it be assumed that $I[c(z + 1)] > 0$, $I[c(z - 1)] > 0$. This is in accord with the conditions of most physical problems wherein z is real and $I(c) > 0$. The contour may now start and terminate at $t = +\infty$. In particular a solution may be represented by

$$w(z) = \int_{\infty}^{(-1+,1+)} e^{icst}\,(1 - t^2)^a\,Se^1_{a,l}\,(t)dt. \quad (5)$$

Let $I_n = \int_{\infty}^{(-1+,1+)} e^{icst}\,(1 - t^2)^a\,T^a_n\,(t)dt.$

Then on replacing T^a_n by the differential expression

$$T^a_n\,(t) = \frac{(-1)^n\Gamma(n + 2a + 1)}{2^{n+a}\Gamma(n + 1)\Gamma(n + a + 1)}\,(1 - t^2)^{-a}\,\frac{d^n}{dt^n}\,(1 - t^2)^{n+a}, \quad (6)$$

and integrating partially one finds

$$I_n = \frac{\Gamma(n + 2a + 1)}{2^{n+a}\Gamma(n + 1)\Gamma(n + a + 1)}\,i^n e^{(n+a)\pi i}(cz)^n \int^{(-1+,1+)} e^{icst}(t^2 - 1)^{n+a}dt. \quad (7)$$

It has been shown by Watson[3] that

$$J_{-n-a-1/2}(cz) = \frac{\Gamma(-n-a)e^{(n+a)\pi i}(cz)^{n+a+1/2}}{2^{n+a+1}\,\pi\sqrt{2\pi}} \int^{(-1+,1+)} e^{iczt}(t^2-1)^{n+a}dt, \tag{8}$$

and hence

$$I_n = \frac{i^n\Gamma(n+2a+1)}{\Gamma(n+1)}(-1)^{l+1}\,2\sqrt{2\pi}\sin a\pi(cz)^{-a-1/2}J_{-n-a-1/2}(cz). \tag{9}$$

Multiply both sides of (9) by d_n^l, replace d_n^l on the right side by a_n^l according to (I-22), and sum over odd or even values of n as l is odd or even. There results

$$w(z) = \int_\infty^{(-1+,1+)} e^{iczt}(1-t^2)^a Se_{a,l}^1(t)dt = 2k_l(-1)^{l+1}\sin a\pi(cz)^{-a-1/2}\sum_n{}' a_n^l J_{-n-a-1/2}(cz). \tag{10}$$

The integral thus evaluated may be readily expressed in terms of the functions $Re_{a,l}^1$ and $Re_{a,l}^2$. In virtue of the definition of the Neumann function $N_{n+a+1/2}$ when n is an integer,

$$N_{n+a+1/2} = -\tan a\pi\, J_{n+a+1/2} - \frac{(-1)^n}{\cos a\pi}J_{-n-a-1/2}, \tag{11}$$

it follows that

$$(-1)^{l+1}(cz)^{-a-1/2}\sum_n{}' a_n^l J_{-n-a-1/2}(cz) = \cos a\pi[\tan a\pi\, Re_{a,l}^1 + Re_{a,l}^2]. \tag{12}$$

In the domain $1 < z \leqslant \infty$ one has therefore

$$\int_\infty^{(-1+,1+)} e^{iczt}(1-t^2)^a Se_{a,l}^1(t)dt = k_l \sin 2a\pi[\tan a\pi\, Re_{a,l}^1 + Re_{a,l}^2]. \tag{13}$$

The contour integral must now be evaluated for values of z lying in domain $-1 < z < 1$. To accomplish this, let $Se_{a,l}^1(t)$ be replaced by $\lambda_l k_l Re_{a,l}^1(ct)$ in the integrand of (13) and consider integrals of the type

$$K_n = \int_\infty^{(-1+,1+)} e^{iczt}(t^2-1)^a(ct)^{-a-1/2}\,J_{n+a+1/2}(ct)dt. \tag{14}$$

The Bessel function may be replaced by its representation as a definite integral,

$$J_{n+a+1/2}(ct) = \frac{(ct)^{n+a+1/2}}{2^{n+a}\sqrt{2\pi}\Gamma(n+a+1)}\int_{-1}^{1} e^{icts}(1-s^2)^{n+a}ds, \tag{15}$$

and on reversing the order of integration there results for (14),

$$K_n = \frac{1}{2^{n+a}\sqrt{2\pi}\Gamma(n+a+1)} \int_{-1}^{1} ds(1-s^2)^{n+a} \int_{\infty}^{(-1+,1+)} e^{ic\iota(z-s)}(t^2-1)^a(ct)^n dt. \quad (16)$$

Let the contour of integration be taken around the points $(-1, +1)$ such that $t > 1$ over the entire path. Then $(t^2-1)^a$ may be expanded in a convergent series of inverse powers of t, and it may be shown[4] that

$$\int_{\infty}^{(-1+,1+)} e^{ic\iota(z-s)}(t^2-1)^a(ct)^n dt = \frac{2\pi e^{-a\pi i - \frac{n\pi i}{2}}}{c^{2a+1}} \sum_{m=0}^{\infty} \frac{(-1)^m\Gamma(m-a)}{m!\Gamma(-a)\Gamma(2m-n-2a)} c^{2m}(z-s)^{2m-2a-n+1}. \quad (17)$$

$$K_n = \frac{1}{2^{n+a}\sqrt{2\pi}\Gamma(n+a+1)} \frac{2e^{-a\pi i - \frac{n\pi i}{2}}(-1)^{l+1}\sin 2a\pi}{c^{2a+1}} \quad (18)$$

$$\times \sum_{m=0}^{\infty} \frac{(-1)^m\Gamma(m-a)\Gamma(n+2a+1-2m)}{m!\,(-a)} c^{2m} \int_{-1}^{1} (1-s^2)^{n+a}(z-s)^{2m-2a-n-1} ds$$

Now by (3) it appears that

$$\int_{-1}^{1} (1-s^2)^{n+a}(z-s)^{2m-2a-n-1} ds = \frac{2^{n+a+1}\Gamma(n+a+1)}{\Gamma(n+2a-2m+1)} e^{-a\pi i} \mathbf{T}^{a-2m}_{n+2m} \quad (19)$$

and on substituting in (18) and reducing, one obtains

$$K_n = 2\sqrt{\frac{2}{\pi}} \frac{e^{-2a\pi i}\sin 2a\pi(-1)^{l+1}}{c^{2a+1}} e^{-\frac{n\pi i}{2}} \sum_{m=0}^{\infty} \frac{(-1)^m\Gamma(m-a)}{m!\Gamma(-a)} c^{2m} \mathbf{T}^{a-2m}_{n+2m}. \quad (20)$$

Finally, in virtue of (14) and (13), there results

$$\sum_n{}' a^l_n K_n = \frac{e^{-a\pi i}}{\lambda_l k_l} \int_{\infty}^{(-1+,1+)} e^{ics\iota}(1-t^2)^a Se^1_{a,l}(t) dt, \quad (21)$$

or

$$\tan a\pi\, Re^1_{a,l} + Re^2_{a,l} = \lambda_l 2\sqrt{\frac{2}{\pi}} \frac{e^{-a\pi i}(-1)^{l+1}}{c^{2a+1}} \sum_n{}' a^l_n e^{-\frac{n\pi i}{2}} \sum_{m=0}^{\infty} \frac{(-1)^m\Gamma(m-a)}{m!\Gamma(-a)} c^{2m} \mathbf{T}^{a-2m}_{n+2m} \quad (22)$$

The right-hand side of (22) is next to be expressed as a multiple of the function $Se^2_{a,l}$ defined in (4). In the simple case $a = 0$ corresponding to axially symmetric spheroidal functions, the summation over m breaks

off with the first term. Replacing a_n^l by d_n^l according to (I-22) yields at once

$$Re_{o,l}^2 = -\frac{2\lambda_l}{c} e^{\frac{i\pi l}{2}} Se_{o,l}^2. \tag{23}$$

In order to determine the proportionality factor, let us write

$$\sum_n{}' a_n^l e^{-\frac{n\pi i}{2}} \sum_{m=0}^{\infty} \frac{(-1)^m \Gamma(m-a)}{m!\Gamma(-a)} c^{2m} \mathbf{T}_{n+2m}^{a-2m} = \mu_l(c) \sum_n{}' d_n^l \mathbf{T}_n^a, \tag{24}$$

and evaluate at the ordinary point $z = 0$. By an obvious modification of Hobson's expressions for the functions Q_{n+a}^a on the cross cut between ± 1[5] we have

$$\mathbf{T}_n^a(o) = -2^{a-1} \sin \frac{n+2a}{2}\pi \frac{\Gamma\left(\frac{n+2a+1}{2}\right)}{\Gamma\left(\frac{n+2}{2}\right)} \sqrt{\pi}, \tag{25}$$

and a corresponding expression for $\mathbf{T}_{n+2m}^{a-2m}(o)$. Introducing these values into (24) one obtains an expression for the joining factor μ_l in terms of the known expansion coefficients.

$$\mu_l(c) \sum_n{}' d_n^l e^{\frac{in\pi}{2}} \frac{\Gamma\left(\frac{n+2a+1}{2}\right)}{\Gamma\left(\frac{n+2}{2}\right)} =$$

$$\sum_n{}' a_n^l \sum_{m=0}^{\infty} \frac{\Gamma(m-a)c^{2m}}{m!\Gamma(-a)2^{2m}} \frac{\Gamma\left(\frac{n+2a-2m+1}{2}\right)}{\Gamma\left(\frac{n+2m+2}{2}\right)}. \tag{26}$$

The analytic connection between the functions of the second kind is therefore

$$\tan a\pi \, Re_{a,l}^1 + Re_{a,l}^2 = 2\sqrt{\frac{2}{\pi}} \frac{(-1)^{l+1} e^{-a\pi i}}{c^{2a+1}} \lambda_l \mu_l Se_{a,l}^2. \tag{27}$$

In case a is an integer, as in the spheroidal wave functions, $Se_{a,l}^2$ represents the analytic continuation of $Re_{a,l}^2$ into the origin. In particular

$$\mu_l = \sqrt{\frac{\pi}{2}} e^{-\frac{\pi l i}{2}}, \quad (a = 0), \tag{28}$$

and

$$\mu_l = \sqrt{\frac{\pi}{2}} e^{-\frac{\pi l i}{2}} \left(\frac{b_l}{2} - \frac{c^2}{2} + 1\right), \quad (a = 1). \tag{29}$$

If a is half an odd integer, as in the case of the Mathieu functions ($a = -1/2$), tan $a\pi$ in (27) becomes infinite. The function $(cz)^{-a-1/2}\sum_n' a_n^l J_{-n-a-1/2}(cz)$, however, is then no longer independent of $Re_{a,l}^1$ and, as might be expected, it is easy to show that $Se_{a,l}^2$ is now proportional to $Se_{a,l}^1$. An independent expression for $Se_{a,l}^2$ applicable in this case may be obtained by a limiting process, the details of which will be published later.

[1] J. A. Stratton, "Spheroidal Functions," *Proc. Nat. Acad. Sci.*, **21**, 51–56 (1935).
[2] E. W. Hobson, *Theory of Spherical and Ellipsoidal Harmonics*, p. 193.
[3] G. N. Watson, *Theory of Bessel Functions*, p. 164.
[4] Whittaker and Watson, *Modern Analysis*, 4th Ed., p. 245.
[5] Hobson, loc. cit., p. 229.

THERMAL EQUILIBRIUM IN A GENERAL GRAVITATIONAL FIELD

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1. *Introduction.*—In the absence of any appreciable gravitational field the conditions satisfied by the temperature of a medium having no thermal flow from one portion to another, are well known to be those of uniformity throughout the system. In the presence of a gravitational field, however, the conditions imposed at thermal equilibrium on the proper temperature—as measured at different points of the medium by local observers—are considerably more complicated, owing to the association of inertia and weight with all forms of energy and the consequent tendency for heat to flow from regions of higher to those of lower gravitational potential. Nevertheless, for the special case of static gravitational fields, these more complicated conditions have already been obtained,[1,2] and it is the purpose of the present article to investigate the conditions for thermal equilibrium in the general case of any kind of gravitational field.

The investigation shows that a reasonably straightforward treatment can be found which leads to an apparently satisfactory, covariant expression of the general conditions for thermal equilibrium. The possible value of such a result can be two-fold. In the first place it satisfies our desire for coherent theory, since our previous expression of the conditions for thermal equilibrium, which applied only to static systems and was made in the non-covariant language of a kind of coördinate system then appropriate, can now be regarded as a special case of the generally covariant expression applying to any kind of system. In the second place, the expression ob-

tained might prove useful if it should sometime become necessary to consider non-homogeneous cosmological models with thermal flow from one portion of the model to another.

2. *Conditions for Thermal Equilibrium in a Static System.*—Let us first consider the case of a static system corresponding to the line element

$$ds^2 = g_{ij}dx^i dx^j + g_{44}dx^4 dx^4 \qquad (i, j = 1, 2, 3) \tag{1}$$

where all the components of the metrical tensor are independent of the time-like coördinate x^4

$$\frac{\partial g_{\mu\nu}}{\partial x^4} = 0 \qquad (\mu, \nu = 1, 2, 3, 4) \tag{2}$$

and the components of the "velocity" of the medium are given by

$$u^i = \frac{dx^i}{ds} = 0, \qquad u^4 = \frac{dx^4}{ds} = (g^{44})^{1/2}. \tag{3}$$

The conditions at thermal equilibrium are then known to be given by the expressions

$$\frac{\partial \log T_0}{\partial x^i} = -\frac{\partial \log (g_{44})^{1/2}}{\partial x^i} \tag{4}$$

$$\frac{\partial \log T_0}{\partial x^4} = 0 \tag{5}$$

where the first equation relates the proper temperature T_0—as measured at different positions by local observers at rest in the medium—to the gravitational potential g_{44}, and the second equation states that the proper temperature at a given position is independent of the time-like coördinate x^4 in accordance with our assumption of a static system.

The above expression for the relation between proper temperature and position in the gravitational field has received a satisfactory derivation by considering the effect of the field on the pressure and hence temperature of black-body radiation in equilibrium with the system,[1] and—for the analytically simple case of a fluid system with spherical symmetry—it has also been justified by direct use of the relativistic form of the second law of thermodynamics.[2] Hence equations (4) and (5) should provide a reliable starting point for further generalization.

3. *Conditions for Thermal Equilibrium in a Static System Reëxpressed in Covariant Language.*—As a start in the process of generalization, we may first reëxpress the above conditions for thermal equilibrium in the case of static systems in the simple form

$$\frac{\partial \log T_0}{\partial x^\nu} = (u_\nu)_\alpha u^\alpha \qquad (\nu, \alpha = 1, 2, 3, 4) \tag{6}$$

where u_ν and u^α refer to the "velocity" of the medium with respect to the coördinates used. This expression can also be written in the more explicit forms

$$\frac{\partial \log T_0}{\partial x^\nu} = g_{\nu\beta}(u^\beta)_\alpha u^\alpha$$

$$= g_{\nu\beta}\left(\frac{\partial u^\beta}{\partial x^\alpha} + \{\alpha\gamma, \beta\}u^\gamma\right)u^\alpha$$

$$= g_{\nu\beta}\left(\frac{\partial u^\beta}{\partial x^\alpha} + \frac{1}{2}g^{\beta\delta}\left\{\frac{\partial g_{\alpha\delta}}{\partial x^\gamma} + \frac{\partial g_{\gamma\delta}}{\partial x^\alpha} - \frac{\partial g_{\alpha\gamma}}{\partial x^\delta}\right\}u^\gamma\right)u^\alpha. \qquad (6')$$

Since equation (6) is seen to be a tensor equation, it will be true in all coördinate systems if true in one, and will hence be a valid covariant expression of the conditions for thermal equilibrium in static systems, if it reduces to the former equations (4) and (5) when expressed in the special coördinates that were then used. In these coördinates, however, we know from (3) that u^γ and u^α will be zero except for γ and α equal to 4, and from the static character of the system in these coördinates we know that all derivatives with respect to x^4 will vanish. Hence our equation in the last form (6'), immediately reduces to

$$\frac{\partial \log T_0}{\partial x^\nu} = -\frac{1}{2}g_{\nu\beta}g^{\beta\delta}\frac{\partial g_{44}}{\partial x^\delta}u^4u^4$$

$$= -\frac{1}{2}g^\delta_\nu\frac{\partial g_{44}}{\partial x^\delta}g^{44} = -\frac{\partial \log (g_{44})^{1/2}}{\partial x^\nu}$$

and with $\nu = 1, 2, 3$ this is equivalent to (4), while that $\nu = 4$ it is equivalent to (5) on account of the static character of the system. Thus equation (6) is shown to be a valid covariant expression of the conditions for thermal equilibrium in static systems.

4. *Conditions for Thermal Equilibrium in a Static System Reëxpressed in Proper Coördinates.*—In accordance with the foregoing, nevertheless, equation (6) is presumably an expression of the conditions for thermal equilibrium only in the case of static systems, and some further physical content may have to be included to obtain an expression applicable to the general case of non-static systems, which will be of such nature that no coördinates can be found that will permit a description of the system by our original equations (1), (2) and (3).

To obtain an idea of the physical content already implied by equation (6), we shall find it profitable to reëxpress its consequences in terms of the proper coördinates (x, y, z, t) which would be appropriate for use by a local observer momentarily at rest with respect to the medium at some selected point and time. In these proper coördinates, the expression for

the line element will reduce in the immediate neighborhood of that point and time to the special relativity form

$$ds^2 = -dx^2-dy^2-dz^2 + dt^2 \tag{7}$$

where the gravitational potentials have their Galilean values and their first derivatives vanish

$$g_{\mu\nu} = \pm 1, 0 \qquad \frac{\partial g_{\mu\nu}}{\partial x^\alpha} = 0. \tag{8}$$

Furthermore, in proper coördinates the components of the "velocity" of the medium will have the simple values

$$u^i = \frac{dx^i}{ds} = 0 \qquad (i = 1, 2, 3) \qquad u^4 = \frac{dt}{ds} = 1. \tag{9}$$

Finally, since we can write the general formula for interval in the form

$$1 = g_{11}\frac{dx}{ds}\frac{dx}{ds} + 2g_{12}\frac{dx}{ds}\frac{dy}{ds} + \ldots\ldots + g_{44}\frac{dt}{ds}\frac{dt}{ds}$$

it is evident that we must have as a consequence of differentiating both sides of this expression the relation

$$\frac{\partial}{\partial x^\alpha}\left(\frac{dt}{ds}\right) = \frac{\partial u^4}{\partial x^\alpha} = 0 \tag{10}$$

at the point of interest, since the differentiation of all terms in the above expression except the last will lead to zero after (8) and (9) are substituted.

Making use of equations (8), (9) and (10) in connection with the conditions for equilibrium given by (6′), we then easily obtain

$$\left.\begin{aligned} \frac{\partial \log T_0}{\partial x} &= -\frac{\partial u^1}{\partial t} = -\frac{\partial u_x}{\partial t} = -\dot{u}_x \\ \frac{\partial \log T_0}{\partial y} &= -\frac{\partial u^2}{\partial t} = -\frac{\partial u_y}{\partial t} = -\dot{u}_y \\ \frac{\partial \log T_0}{\partial z} &= -\frac{\partial u^3}{\partial t} = -\frac{\partial u_z}{\partial t} = -\dot{u}_z \end{aligned}\right\} \tag{11}$$

$$\frac{\partial \log T_0}{\partial t} = 0 \tag{12}$$

as an expression of the conditions for thermal equilibrium in a static system using proper coördinates for the selected point and time, where it will be noted that $\dot{u}_x$, $\dot{u}_y$, $\dot{u}_z$ are the components of the acceleration of the medium

as it would be observed and measured in the ordinary manner by the local observer at the point of interest.

This result makes the physical implications of our present conditions for thermal equilibrium fairly clear. Equations (11) show that the local freely falling observer—for whom gravitational effects have been abolished—will find a temperature gradient oppositely directed to the acceleration which he observes for the medium, and in accordance with the principle that all forms of energy have inertia will interpret this as the necessary condition to prevent heat from "lagging behind" in the accelerated medium. Equation (12) on the other hand merely states that the temperature at a given point in the medium is not observed to be changing with the time in agreement with our assumption of a static system.

5. *Conditions for Thermal Equilibrium in a General System.*—The above result also makes clear what further physical content should be included in order to treat non-static as well as static systems. Again considering proper coördinates for a particular point in the medium, it seems evident from the above mentioned notions as to the relation between energy and inertia that equations (11) should still be retained as the conditions to prevent thermal flow through the medium. On the other hand, it also appears evident that equation (12) is too restrictive for the general case, since in a non-static system even in the absence of heat flow the temperature at a given point in the medium might be changing with proper time, owing to the local "generation of heat" by a variety of processes of which adiabatic compression would be a simple example.

These considerations then immediately suggest, as a tensor expression of the general conditions for the absence of thermal flow in any kind of system, the equation

$$\frac{\partial \log T_0}{\partial x^\nu} = (u_\nu)_\alpha u^\alpha + u_\nu \frac{d \log T_0}{ds}$$

$$= g_{\nu\beta}\left(\frac{\partial u^\beta}{\partial x^\alpha} + \{\alpha\gamma, \beta\} u^\gamma\right) u^\alpha + g_{\nu\beta} u^\beta \frac{d \log T_0}{ds}. \qquad (13)$$

With the help of equations (8), (9) and (10), this expression is easily seen to reduce in proper coördinates at the point of interest for the cases $\nu = 1, 2, 3$ to equations (11), and for the case $\nu = 4$ to the identity

$$\frac{\partial \log T_0}{\partial t} = \frac{d \log T_0}{ds} \qquad (14)$$

which allows for any arbitrary changes in the local temperature of the medium which actually do take place for causes other than the flow of heat. Since equation (13) is a covariant expression its validity in proper co-

ordinates secures its validity in all coördinates, and we may hence regard it as a satisfactory expression of the conditions for thermal equilibrium in a general gravitational field.

[1] Tolman and Ehrenfest, *Phys. Rev.*, **36**, 1791 (1930).
[2] Tolman, *Ibid.*, **35**, 904 (1930).

ON THE EXPANSION OF GREEN'S FUNCTION

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In the case of a number of inhomogeneous partial differential equations the appropriate Green's function is known, and this knowledge gives a theoretical means of solving the equation; though in practice not much can be done unless an expansion of the Green's function is known. It is the purpose of the present paper to indicate a general method for finding such expansions.

This is possible only if the differential equation is separable; for this reason some specification as to its form is essential. Confining our interest to scalar equations of mathematical physics, we note that all of these which consider isotropic media in Euclidian space, and are of order less than four in the space derivatives, necessarily involve Laplace's operator and can be written in the form

$$\nabla^2\varphi + f\left(x_1x_2x_3\frac{\partial}{\partial t}\right)\varphi = \rho(x_1x_2x_3t). \tag{1}$$

When the h_i that determine the line element

$$ds^2 = h_1^2dx_1^2 + h_2^2dx_2^2 + h_3^2dx_3^2 \tag{2}$$

have certain functional forms and f satisfies certain conditions, the homogeneous equation corresponding to (1) can be separated.[1] We show here that the problem of fitting together the resulting solutions so as to form a solution of (1) which satisfies the same boundary conditions at infinity as the Green's function can be made to depend on the solution by Green's method of a single inhomogeneous total differential equation. This last is quite easy and it remains only to compare the solution of (1) thus found with that obtained from the Green's function and so to find the desired expansion.

In showing how the above described process can be carried out we will give the analysis for Poisson's equation in three variables; the principle

is the same in all other cases. Some of the points in connection with other equations which may not be obvious are discussed in a later paragraph. We start with the equation

$$\nabla^2\varphi = 0. \tag{3}$$

On separation this yields three total differential equations of the Sturm-Liouville type; two of these, say those in x_1, x_2, determine as eigen values the two separation constants, say λ_1, λ_2. We write the solutions of (3) in the form

$$\begin{aligned} \varphi &= X_\lambda(x_1, x_2)U_\lambda(x_3) \\ \varphi &= X_\lambda(x_1, x_2)V_\lambda(x_3). \end{aligned} \tag{4}$$

Here $X_\lambda(x_1, x_2)$ is a product of a function of x_1 and a function of x_2 and λ includes both λ_1 and λ_2. $U_\lambda(x_3)$ and $V_\lambda(x_3)$ are two independent solutions of the equation which is not an eigen value equation. More exact specification as to the choice of U_λ and V_λ will be given presently.

We will be specially interested here in the density functions occurring in the orthogonality relations between the eigenfunctions and in the Wronskian of the two solutions of the equation in x_3. Thus if the equations are written in the form

$$\frac{d}{dx_i}\left[p_i(x_i)\frac{dy}{dx_i}\right] + q_i(x_i)y + \lambda_i r_i(x_i)y = 0, \tag{5}$$

we need to know $r_1(x_1)$, $r_2(x_2)$ and $p_3(x_3)$.

If now we set $\rho = 0$ except on the surface determined by holding constant the coördinate which occurs in the equation which is not an eigen value equation, we can easily build up a solution of the inhomogeneous equation from the solutions of the homogeneous equation. In our case this coordinate is x_3, and we let

$$\begin{aligned} \nabla^2\varphi &= 0 && x_3 < x_3' \\ \nabla^2\varphi &= a_\lambda X_\lambda(x_1x_2) && x_3' < x_3 < x_3' + \Delta x_3' \\ \nabla^2\varphi &= 0 && x_3 > x_3'. \end{aligned} \tag{6}$$

We have only to join the two solutions at $x_3 = x_3'$ and choose the constant factor so that the change in the x_3 component of $\nabla\varphi$ will have the proper value. The result is

$$\begin{aligned} \Delta\varphi &= \frac{p_3(x_3')h_3^2(x_3')V_\lambda(x_3')}{c_\lambda} a_\lambda \Delta x_3' U_\lambda(x_3)X_\lambda(x_1x_2) && x_3 > x_3' \\ \Delta\varphi &= \frac{p_3(x_3')h_3^2(x_3')U_\lambda(x_3')}{c_\lambda} a_\lambda \Delta x_3' V_\lambda(x_3)X_\lambda(x_1x_2) && x_3 < x_3' + \Delta x_3'. \end{aligned} \tag{7}$$

The Wronskian which enters in the process of adjusting the discontinuity in derivative has been replaced by c_λ/p_3. Clearly the above is nothing but the use of Green's method on the inhomogeneous equation in x_3.

Now that we have seen how the functions are to be used, we can tell how U_λ and V_λ are chosen from among the infinity of possible sets of independent solutions. Each must be finite in the interval in which it is taken and $U_\lambda X_\lambda$ must have a behavior at infinity similar to that of the Green's function whose expansion is desired.

Using the solution (7) and the orthogonality properties of X_λ, we find by integration the solution of the general inhomogeneous equation. Thus let

$$\nabla^2\varphi = \rho(x_1x_2x_3) = \sum_\lambda a_\lambda(x_3)X_\lambda(x_1x_2), \tag{8}$$

where

$$a_\lambda(x_3) = \frac{1}{N_\lambda^2}\int\int \rho(x_1x_2x_3)X_\lambda(x_1x_2)r_1(x_1)r_2(x_2)dx_1dx_2 \tag{9}$$

with N_λ^2 the integral of $X_\lambda^2 r_1 r_2$.

Then

$$\varphi = \sum_\lambda \frac{1}{N_\lambda^2}\int\int\int U_\lambda(x_3)X_\lambda(x_1x_2)V_\lambda(x_3')X_\lambda(x_1'x_2')\rho(x_1'x_2'x_3')$$
$$\frac{p_3(x_3')h_3^2(x_3')r_1(x_1')r_2(x_2')}{c_\lambda}dx_1'dx_2'dx_3' \qquad x_3 > x_3'. \tag{10}$$

This is certainly a solution of the inhomogeneous equation; and, if U_λ and V_λ have been properly chosen, it is not necessary to add a solution of the homogeneous equation to make this solution identical with that obtained from the Green's function. Thus we could obtain the desired expansion if we understood the behavior of the factor $p_3h_3^2r_1r_2$. Using the fact that the $V_\lambda X_\lambda$ are solutions of Laplace's equation and that they form an orthogonal set, it can be shown that the above quantity is equal to $bh_1h_2h_3$ with b a constant. Thus, if G is the Green's function*

$$G = \sum \frac{b}{C_\lambda N_\lambda^2} U_\lambda(x_3)X_\lambda(x_1x_2)V_\lambda(x_3')X_\lambda(x_1'x_2') \qquad x_3 > x_3'. \tag{11}$$

We can summarize as follows the general methods illustrated in connection with Poisson's equation. The homogeneous partial differential equation is separated into total differential equations: if there are n variables there result n total differential equations, $n-1$ of which are eigen value equations determining the separation constants. The remaining equation, say that in x_n, has a number of independent solutions equal to its order. Products of solutions of the eigen value equations form a complete orthogonal set in terms of which we expand the ρ of equation (1); the expansion coefficients are functions of x_n. Considering only that part of ρ in the shell

between x_n' and $x_n' + dx_n'$ and of this only one term in the orthogonal expansion, we show that a solution of the inhomogeneous equation can be built from solutions of the homogeneous equation; thus, if the equation (1) is of the second order in x_n, we combine solutions so as to obtain a solution which is continuous but has a discontinuity in first derivative: this is possible since there will be two independent solutions of the total differential equation in x_n. From such solutions we find the general solution of (1) by summing all the terms in the orthogonal expansion and integrating over x_n'. With proper choice of the functions of x_n used, the solution so determined is identical with that obtained by using the known Green's function; and so, since ρ is arbitrary, the desired expansion is determined.

There follow a few expansions obtained by the above method and chosen so as to illustrate some points of interest. Thus (12) and (13) are two different expansions of $\frac{e^{ikr_{12}}}{r_{12}}$ in cylindrical coördinates. In this case there is a choice as to which are to be considered as the eigenvalue equations; with the result that two different expansions are possible. We note in connection with (13) that, in order that the Bessel's functions form a complete set, $\sqrt{k^2 + m^2}$ must vary from zero to infinity; for this reason the integration over m is from ik to infinity. Another case where several expansions are possible is illustrated in (14) where one of the possible expansions of the retarded potential solution of the wave equation is given. Here the space equations are considered as eigenvalue equations determining the wave number k, and one uses zero and sin $kc(t-t')$ as the time part of the solutions before and after t'. It is also possible to consider the time equation as determining k and use half order Bessel and Hankel functions in the radial part of the solution for r less than and greater than r'.

Finally (15), which is an expansion of a Green's function for the diffusion equation, illustrates a case where the equations are of the first order in x_n, and one introduces a discontinuity in value of the function of x_n.

$$\frac{e^{ikr_{12}}}{r_{12}} = \frac{i}{2}\sum_n \int_{-\infty}^{+\infty} J_n(\sqrt{k^2 - m^2}\, r_1)\, H_n^{(1)}\, (\sqrt{k^2 - m^2}\, r_2)\, e^{i(m(z_2 - z_1) + n(\varphi_2 - \varphi_1))}\, dm \qquad r_2 > r_1 \tag{12}$$

$$\frac{e^{ikr_{12}}}{r_{12}} = \sum_n \int_{ik}^{\infty} e^{-m(z_2 - z_1)}\, J_n(\sqrt{k^2 + m^2}\, r_1)\, J_n(\sqrt{k^2 + m^2}\, r_2)\, e^{in(\varphi_2 - \varphi_1)}\, dm \qquad z_2 > z_1. \tag{13}$$

If

$$G = c\sum_{l,m} \frac{(2l+1)\,(l-m)!}{(l+m)!} \int_0^{\infty} \frac{1}{\sqrt{kr_1}}\, J_{l+1/2}\,(kr_1)\, \frac{1}{\sqrt{kr_2}}\, J_{l+1/2}(kr_2) P_l^m(\theta_1) P_l^m(\theta_2) e^{im(\varphi_2 - \varphi_1)} \sin ck(t_2 - t_1) kdk \quad t_2 > t_1 \tag{14}$$

then

$$\int \frac{\rho(r_1, \theta_1, \varphi_1, t_2 - r_{12}/c)}{r_{12}} d\tau_1 = \int\int G\rho(r_1\theta_1\varphi_1 t_1) d\tau_1 dt_1$$

$$\frac{1}{(2a\sqrt{\pi t})^3} e^{-r_{12}^2/4a^2(t_2 - t_1)} = \frac{1}{4\pi} \sum_{l,m} \frac{(2l+1)\,(l-m)!}{(l+m)!}$$

$$P_l^m(\theta_1) P_l^m\ (\theta_2) e^{im(\varphi_2 - \varphi_1)} \int_0^\infty \frac{1}{(kr_1)^{1/2}} J_{l+1/2}(kr_1) \frac{1}{(kr_2)^{1/2}} J_{l+1/2}(kr_2)$$

$$e^{-k^2a^2(t_2 - t_1)}\, k^2 dk \qquad t_2 > t_1. \tag{15}$$

[1] L. P. Eisenhart, *Ann. Math.*, **35**, 284 (1934); L. P. Eisenhart, *Phys. Rev.*, **45**, 427 (1934).

[2] A. Sommerfeld, *Ann. d. Physik*, **28**, 665 (1909).

STUDIES OF CAROTENOID PIGMENTS IN FISHES. III. THE EFFECTS OF INGESTED CAROTENOIDS UPON THE XANTHOPHYLL CONTENT OF FUNDULUS PARVIPINNIS

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Our earliest experiments along these lines (Sumner and Fox, 1933) indicated that in *Fundulus parvipinnis* the proportional amount of the xanthophyll in the body underwent little or no change in fishes kept 6 or 7 weeks in the laboratory and fed upon the local red beach-worm (*Thoracophelia* sp.). These experiments likewise gave no evidence that the proportion of xanthophyll was altered by the sojourn of these fishes upon backgrounds of different colors or shades, even though these last were potent in influencing the visible color of the animals. At the same time, it was shown for another fish, *Girella nigricans*, that on the one hand the proportional amount of xanthophyll was greatly reduced under laboratory conditions and, on the other hand, the rate of this reduction was greatly influenced by the background.

In a later series of experiments with *Girella* (Sumner and Fox, 1935) the authors confirmed both of the foregoing conclusions, the loss of xanthophyll in captivity and the influence of the background upon the rate of this loss. But certain feeding experiments conducted with this object threw no light upon the source of the pigment. Neither the addition of xanthophyll nor of carotene to the fish-meat which was used as food, nor the inclusion in the dietary of an abundance of certain green algae, led

to any differences in the end result. As compared with the proportions of xanthophyll in freshly caught specimens, all of these experimental lots were found to have lost this pigment at approximately equal rates.

An unpublished experiment conducted by us with *Fundulus* in 1934 showed that within a period of three months (Feb. 14 to May 14) the proportional amount of xanthophyll in the fishes remained virtually constant. Since the mean weight of the animals approximately doubled during this period, it is obvious that the absolute amount of xanthophyll must have increased in the same ratio. (Parenthetically, it must be remarked that *Fundulus* appears to possess no carotene in its tissues.)

In this experiment we fed the fishes entirely upon the worm *Thoracophelia*, already referred to. Since this worm was known to contain an abundance of carotene but no detectable amount of xanthophyll (Sumner and Fox, 1933), it might have been concluded that the former carotenoid had been converted by the fishes into the latter. It was found, however, that the walls of the cement tank used in our experiment bore a heavy coating of green algae, and furthermore, that the fishes were feeding freely upon this, as was evident from the composition of their feces. It seems worth while, nevertheless, to record the results for this series of fishes (table 1).

TABLE 1

XANTHOPHYLL CONTENT OF MALE AND FEMALE *Fundulus*, DETERMINED THROUGHOUT A PERIOD OF THREE MONTHS[1]

DATE 1934	NO.	MEAN WT. (GMS.)	XANTHOPHYLL CONCENTR. (MG. PER 100 GM.)	ABSOLUTE XANTHOPHYLL CONTENT PER FISH (CALCULATED)
Feb. 14	6♂	4.8	0.80	0.038
	5♀	4.4	0.70	0.031
Mar. 3	5♂	5.1	0.88	0.045
	5♀	5.0	0.92	0.046
Apr. 5	5♂	7.1	0.81	0.058
	5♀	5.6	0.95	0.053
Apr. 19	5♂	7.3	0.81	0.059
	5♀	7.1	0.83	0.059
May 4	5♂	8.5	0.76	0.065
	5♀	7.2	0.77	0.055
May 15	5♂	9.0	0.80	0.072
	6♀	9.4	0.78	0.073

The question whether animals have the power to synthesize carotenoids has been variously answered, but the prevailing belief seems to be that these substances must be derived directly or indirectly from plants. Some experiments with mammals and birds by Palmer and others (Palmer, 1922, and earlier) furnish strong evidence for the derivation of carotene and xanthophyll from vegetable foods. They furthermore seem to indicate

that either of these substances, when found in the tissues of such animals, must have been ingested *as such*, and that they are not subject to conversion, one into the other, either in the animal body or *in vitro*.

On the other hand, it has recently been suggested (Lönnberg, 1932) that in fishes one type of carotenoid may be transmuted into another showing shorter wave-lengths, in the process of digestion.[2] The same writer makes the significant comment (1931, p. 11):

"The preponderance of the carotinoids of the xanthophyll group among the fishes is of great interest considering, that among the marine invertebrates, which serve them as food, those of the carotine group are by far more numerous than those of the other."

It is the principal object of the present paper to report upon a new series of experiments, designed to test the two questions raised above, namely: first, whether any fishes can produce xanthophyll when supplied with a diet free from all carotenoids, and, second, whether they have the power to convert carotene into xanthophyll. We may anticipate our findings by answering the first question in the negative, the second (provisionally), in the affirmative.

The species employed for this purpose was the Pacific killifish (*Fundulus parvipinnis* Girard). Of these there were nine lots of about 25 each. Each lot was placed in a separate aquarium, 16 × 10 × 10 inches in size. The fishes of three of these aquaria received the chopped flesh and skin of the garibaldi (*Hypsypops rubicunda* (Girard)); those of the second group of aquaria received the beach-worms already referred to; those of the third lot the chopped white meat of the California "halibut" (*Paralichthys californicus*). The experimental fishes were so selected according to size that the three lots receiving the same diet comprised "large," "medium" and "small" individuals, respectively.

The fishes were fed with regularity three times a week. An excess of the food substance was placed in each aquarium and the residue was commonly siphoned off before the end of the day. Our stock, as a whole, remained in excellent physical condition throughout the entire experiment. Among the 229 fishes, there were only three deaths during the last ten weeks, all of these occurring in the same aquarium.

The garibaldi is a brilliant orange-red fish, containing large amounts of xanthophyll in its superficial tissues. The beach-worm, as before stated, contains large amounts of carotene, but (so far as detected) no xanthophyll. The white flesh of the "halibut," taken in small quantities, yields no appreciable amount of pigment of any sort. When 200 grams of the flesh were extracted, it was found that some 0.008 mg. of carotene was present. Since the total amount of halibut flesh consumed by the *Fundulus* during the three months of the experiment did not exceed 700 grams, we may set the amount of carotene available from this source as approximately

0.028 mg., or slightly more than one per cent of the amount of carotenoid (xanthophyll) present in this lot of fishes (table 2). It is obvious that we may, for the purposes of this experiment, regard the halibut flesh as carotenoid-free.

There were constant and interesting differences in the quantity and quality of the feces from these differently fed lots of fishes. The quantity was greatest for the garibaldi-fed fishes, probably because of the greater proportions of scales and other indigestible matters present in their food. Likewise, the color of the evacuated materials was much more evident, ranging from yellow to bright red. The fecal matter of the worm-fed fishes consisted in part of long white strings, in part of nearly black flocculent matter. That of the halibut-fed group was slight in amount and nearly pure white.

The feeding experiment was commenced on Nov. 26, 1934, and the fishes were weighed, killed and subjected to extraction during the week from February 27 to March 6, 1935. It would have been preferable had we weighed them at the commencement of the differential feeding, but this was done for the first time on December 21, when the occupants of each of the nine aquaria were weighed separately. The final weighing at the time of killing was 71 days (on the average) later. The total increase in weight during this period was computed for each of the nine lots, and from this the rate of increase was derived, expressed as a percentage of the original weight. On the assumption that this rate was uniform throughout the period, we may, by extrapolation, ascertain the probable weight of each lot on November 26. These figures are included in one column of our table. They are believed to be sufficiently accurate for present purposes.

In every case, an interval of two days was allowed to elapse between the last previous feeding of the fishes and the removal of a sample for analysis. We had supposed that this would allow a sufficient time for the voiding of any food remains in the intestine. This assumption was found to be not altogether warranted, as will appear later.

The technique employed before and during the extraction of the pigment and the qualitative tests for distinguishing the carotenoids, as well as our colorimetric procedure, followed rather closely that outlined in our first paper, already referred to (1933). The one important innovation was the use, throughout the present experiments, of a bronze ball-mill,[3] in place of the mortar-and-pestle formerly employed. This naturally resulted in a great saving of labor, as well as in a more thorough comminution of the material.

At the commencement of the present investigation, determinations were made of a number of samples, taken from the same stock as those to be used in the feeding test.[4] Five such samples, consisting of six fishes each, and ranging in mean weight from 2 to 7 grams, gave xanthophyll

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RESULTS OF FEEDING EXPERI-

FOOD	SAMPLE NO.	NO. OF FISHES IN SIZE-GROUP	WEIGHTS OF SIZE-GROUPS COMPUTED AS OF NOV. 26	WEIGHTS OF SIZE-GROUPS, DEC. 21	NO. IN EACH SAMPLE	WEIGHTS OF SAMPLES AT TIME OF KILLING	FINAL WEIGHTS OF SIZE-GROUPS	MEAN WEIGHTS OF FISHES	PERCENTAGE INCREASE IN WEIGHT THROUGHOUT EXPERIMENT
G Garibaldi-fed fishes (entire)	101	25	113.0	116.7	8 ♀	41.7	128.8	5.15	14
	102				8 ♂	45.9			
	103				9	41.2			
	201	25	72.1	74.7	8	27.4	82.9	3.32	15
	202				8	28.4			
	203				9	27.1			
	301	23	35.1	35.9	8	14.5	38.3	1.67	9
	302				8	11.2			
	303				7	12.6			
Garibaldi-fed fishes (minus viscera)	1								
	2								
	3								
W Worm-fed fishes	101	26	118.6	127.6	8 ♀	51.2	157.8	6.07	33
	102				8 ♂	53.2			
	103				10	53.4			
	201	24	71.0	75.8	8	32.8	93.7	3.90	32
	202				8	31.3			
	203				8	29.6			
	301	25	43.8	47.2	8	20.0	60.0	2.40	37
	302				8	21.7			
	303				9	18.3			
H Halibut-fed fishes	101	26	119.1	127.1	8 ♀	46.8	153.7	5.91	29
	102				8 ♂	50.8			
	103				10	56.1			
	201	26	70.9	75.3	8	30.5	89.3	3.43	26
	202				8	26.7			
	203				10	32.1			
	301	26	38.0	40.1	8	15.0	48.2	1.85	27
	302				8	15.2			
	303				10	18.0			

values which agreed very closely with one another. These values were 0.97, 0.95, 1.01, 0.97 and 1.00 mg., respectively, per 100 grams of body weight, the mean (unweighted) being 0.980. This last figure is the starting point for our computations of the changes undergone by the different lots of fishes as a result of the experimental feeding.

The data revealed by the present experiments are set forth in table 2.

BLE 2

MENTS WITH *Fundulus*, 1934–1935

READINGS WITH BLUE-VIOLET FILTER	XANTHOPHYLL (MG. PER 100 GMS. BODY WT.)	MEAN OF EACH SIZE-GROUP	GRAND AVERAGES (WEIGHTED BY WEIGHT)	GAIN OR LOSS IN XANTHOPHYLL CONCENTRATION (PERCENTAGES)	ABSOLUTE AMOUNTS OF XANTHOPHYLL IN EACH SAMPLE (MG.)	TOTAL VALUES FOR EACH SIZE-GROUP	ESTIMATED TOTAL VALUES AT COMMENCEMENT OF EXPERIMENT	GAIN OR LOSS IN ABSOLUTE AMOUNTS OF XANTHOPHYLL	MEAN RATES OF CHANGE
14.10	1.23				0.513				
17.75	1.04	1.14			0.477	1.464	1.107	+32.2	
15.60	1.15				0.474				
20.20	0.94				0.258				
13.45	1.26	1.17	1.162	+19	0.358	0.971	0.707	+37.3	+34.2
12.60	1.31				0.355				
17.50	1.05				0.152				
13.70	1.24	1.22			0.139	0.462	0.344	+34.3	
11.80	1.36				0.171				
						1.336	1.107	+20.6	
			1.061	+ 8		0.886	0.707	+25.3	+22.5
						0.422	0.344	+22.7	
26.35	0.77				0.394				
25.05	0.80	0.81			0.426	1.274	1.162	+ 9.7	
23.25	0.85				0.454				
24.30	0.82				0.269				
21.50	0.90	0.88	0.837	−15	0.282	0.820	0.696	+17.8	+13.5
21.20	0.91				0.269				
23.55	0.84				0.168				
23.05	0.85	0.84			0.184	0.502	0.429	+17.0	
23.90	0.82				0.150				
30.45	0.68				0.318				
27.20	0.75	0.74			0.381	1.137	1.167	− 2.6	
25.80	0.78				0.438				
28.00	0.73				0.223				
26.95	0.75	0.76	0.766	−22	0.200	0.680	0.695	− 2.1	+0.1
24.90	0.80				0.257				
27.15	0.75				0.112				
25.10	0.80	0.86			0.122	0.419	0.372	+12.7	
18.00	1.03				0.185				

The first point of interest revealed by this table is the difference in the growth rates of the three series which have been differently fed. Thus, the garibaldi-fed series shows the least gain in weight during these fourteen weeks (9 to 15 per cent), while the worm-fed series shows the greatest gain (32 to 37 per cent), the halibut-fed series holding an intermediate position in this regard (26 to 29 per cent).

Of chief interest, however, are the figures indicating gains or losses of xanthophyll. Before proceeding, it should be remarked that no attempt was made, except with the largest lots of fishes (101, 102 and 103, of each series) to determine the sex. This was owing partly to the virtual impossibility of determining the sex of younger individuals before killing, but chiefly to the fact that no significant sexual differences in xanthophyll content have been found by us until an advanced stage of the breeding season (cf. tables 1 and 2).

Considering first the garibaldi-fed series, we have two sets of figures depending upon whether or not the xanthophyll extracted from the viscera has been included in the total. Here an explanation is necessary. It was found, early in our studies of the carotenoids of fishes, that the viscera (except for the ovaries of mature females) contained no appreciable amounts of carotenoids. This was true, at least, when the alimentary canal was empty. Preliminary observations led us to believe that the last remnants of food were voided within two days. Hence, in the present experiments we continued our standard procedure of killing the fishes for extraction two days after the last feeding.

However, certain unexpectedly high values in the garibaldi-fed lots raised the suspicion that fragments of the highly colored skin of this fish had been retained in the intestine. A separate analysis of the viscera of the last five samples of the garibaldi-fed fishes confirmed this suspicion. The amount of xanthophyll here found was nearly nine per cent (8.7%) of the total amount found in the entire body. This was not the case with the worm-fed and halibut-fed fishes, the viscera of which yielded in no case more than a scarcely perceptible trace of color.

It seems fairer, therefore, to regard the figures for the garibaldi-fed fishes, "minus viscera," as being more truly representative of the actual xanthophyll content of these fishes, and hence comparable with the yield from the fishes of the other series. We have therefore reduced the mean value obtained for the xanthophyll concentration of this series (1.162) by 8.7 per cent. The value, thus corrected, is 1.061, which is about 8 per cent greater than the presumed xanthophyll concentration of these fishes at the outset. If the mean value for this series, "including viscera," were considered, the indicated increase would be 19 per cent.

These figures denote the *concentration* of xanthophyll in the fishes, i.e., the amount per 100 grams of body weight. It is of interest that this proportional amount seems to have actually increased somewhat during the fourteen weeks of the experiment, despite an increase of from 9 to 15 per cent in the weight of the animals. However, we must not lay too much stress upon this relatively slight increase in a series displaying such considerable variability.

Of much more significance is the fact that a far greater increase has

occurred in the *absolute amounts* of xanthophyll present. To determine these last, each of the figures for xanthophyll concentration has been multiplied by the weight of the given lot of fishes at the time of killing. In order to allow for the probable presence of xanthophyll in the gut-content, the same correction (8.7%) has been applied as before.

The magnitude of this increase in the total xanthophyll present is evident when we compare these values for absolute xanthophyll content, in each lot of fishes, with the presumed content at the commencement of the experiment. The latter figures are obtained by multiplying the initial weight of each lot[5] by the original xanthophyll concentration (0.98).

Comparison of the two columns for total values, near the right-hand side of the table, shows that the increases during the fourteen-week period range from 20.6 per cent to 25.3 per cent in the three size-groups. The weighted mean of these figures is 22.5 per cent. If the crude figures, "including viscera," were employed, the gain would average 34.2 per cent.

Thus, on a diet rich in xanthophyll, the fishes appear to have gained, during the fourteen weeks of our experiment, about eight per cent in the xanthophyll concentration of their tissues, while they gained more than twenty-two per cent in the absolute amount of xanthophyll present.

Even thus, it is interesting to note, the xanthophyll concentration of these fishes at the termination of our feeding experiment, falls considerably below that obtained from three lots of freshly caught (i.e., "wild") *Fundulus* which were tested at the same time. The respective figures are 1.06 and 1.25.

Passing to the halibut-fed series, we find a mean xanthophyll concentration, in the bodies of these fishes, of 0.766 milligrams per hundred grams. This figure is 28 per cent lower than that for the garibaldi-fed series ("minus viscera"). It also represents a reduction of 22 per cent, as compared with the presumed original concentration of 0.980.

Considering the absolute xanthophyll values, the total amount for the halibut-fed series is found to be more than 15 per cent lower than that for the garibaldi-fed series ("without viscera"), despite the fact that the former exceeds the latter by more than 16 per cent in weight.

Considering the rather large reduction (22 per cent) in the xanthophyll concentration of this series, it is of interest to find that the absolute amount of xanthophyll in these fishes has remained nearly constant. Thus, while no new xanthophyll appears to have been acquired by these fishes upon a carotenoid-free diet, that originally present appears to have been retained nearly or quite intact.

Before leaving this series, attention must be called to the occurrence of one thoroughly aberrant determination in the halibut-fed lot. The last sample (H 303) gave a value of 1.03 for xanthophyll concentration and 0.185 for absolute xanthophyll content. While there are circumstances

connected with this sample which lead us to suspect that these values considerably overrate its xanthophyll content, we believe it to be a dangerous practice to omit figures which do not yield "expected" results.[6] However, the omission of this sample would merely add slightly to the already strong evidence for a loss in the relative xanthophyll content, the mean value falling from 0.766 to 0.751. On the other hand, there would now be a slight absolute loss (2.1%), though such a difference could hardly be regarded as significant.

The worm-fed series yielded a fairly consistent set of figures. The mean xanthophyll concentration (weighted) was 0.837 mg. per 100 grams of body weight, a value lying between those for the two other series considered, though considerably nearer that for the halibut-fed lot. In comparison with the presumed initial xanthophyll concentration of 0.98 mg. per 100 grams, there has been a loss of about 15 per cent.

Passing to the absolute xanthophyll content, we meet with some highly significant figures. For each of the three size-groups, there has occurred a marked increase in the amount of xanthophyll present, these gains ranging from 9.7 per cent to 17.8 per cent, the mean (weighted) being 13.5 per cent.[7]

Since this increase in the total amount of xanthophylls has occurred in a series of fishes whose diet is known to contain an abundance of carotene, but is likewise believed to contain no significant amount of xanthophylls, we seem justified in accepting the provisional conclusion that these fishes have in some way converted the carotene into one or more carotenoids of the xanthophyll group.[8] The conversion, chemically speaking, might seem to be a simple one, since xanthophyll ($C_{40}H_{56}O_2$) differs from carotene ($C_{40}H_{56}$) only by the presence of two atoms of oxygen. However, so far as we know, no animal has hitherto been observed to effect this transformation.

Since such a conclusion as the foregoing is not warranted unless we are able to exclude the presence of xanthophyll in the food which was administered to these fishes, we have considered this last question carefully. The small amounts of phytoplankton contained in the sea-water cannot be appealed to in this connection, firstly because *Fundulus* is not a plankton feeder, but chiefly because no such increase in xanthophyll was found to occur in the halibut-fed lot.[9] As already stated, the walls and bottom of all the aquaria were scoured with sufficient frequency to prevent the accumulation of algae.

The only possible source of xanthophyll would seem to be the intestinal contents of the worms upon which this lot of fishes was fed. Previous tests by ourselves[10] failed to reveal the presence of xanthophyll, when moderate weights of these worms were extracted. Owing to the importance of this question, a more searching test was made in connection with the present experiments. For this purpose, 500 grams of the worms (dried

somewhat by "blotting" briefly on paper toweling) were employed. This was roughly a fourth of the amount consumed by these fishes during the fourteen-week period. They were ground in methyl alcohol in the ball-mill and extracted successively with methyl alcohol and ligroin (petroleum ether), according to our usual procedure. After evaporation of the solvent, the fatty constituents in the residue of lipoids and carotenoids were saponified in the customary alcohol-KOH solution, and the product extracted again with ligroin. The resulting ligroin solution was yellow in color, and obviously contained one or more carotenoids. Were these solely members of the carotene group?

In several tests, 50 cc. of this solution were filtered through powdered $CaCO_3$ columns, as described in our 1933 paper. The fact that the filtrate was colored and that no visible amount of the solute seemed to be adsorbed upon the calcium carbonate, showed that the greater part, at least, of the color was due to a carotene rather than a xanthophyll.

On the other hand, the solution was slightly paler after passing through the calcium carbonate than before, and a photometric test indicated an apparent loss of about 10 per cent in the carotenoid content. Since, however, no perceptible amount of color could be extracted from the filter by subsequent washing with a mixture of petroleum ether and alcohol, it is unlikely that xanthophyll was present there. In the absence of facilities for a spectrographic study of these solutions[11] we cannot carry this inquiry further at the present time.

The three italicized passages (pp. 337–338) should serve as a sufficient summary.

[1] Some of the females in April and May contained mature eggs.

[2] It is even contended by Verne (1926) that certain animals are able to synthesize carotenoids. Zechmeister (1934) likewise concedes this possibility.

[3] This was the "Turnip-Bowl Ball-Mill," manufactured by Paul O. Abbé, Incorporated, Little Falls, New Jersey.

[4] This stock had been caught from two to three weeks earlier, and fed, during this interval, upon worms.

[5] In this case, not the ultimate lot or "sample," but the entire collection of fishes in each aquarium.

[6] No determinations have been rejected throughout the present experiments with the exception of two of those which were made at the commencement of the feeding. These two gave what we regard as altogether impossible values of xanthophyll for these fishes, the values being only 40 per cent of the mean for the other five determinations. Not only do the latter agree closely among themselves (see pp. 333–334), but their mean is almost identical with the mean of our determinations of 14 sample lots of *Fundulus* made at this same time of the year in 1933 (Sumner and Fox, 1933). With respect to one of the aberrant samples here referred to, we had at the time rather definite evidence of xanthophyll loss following extraction.

[7] This comparison of totals for the three size-groups may not, in itself, appear very convincing. It would have been desirable, of course, to have comparable values for each of the three samples in each size-group, thus permitting of nine comparisons

instead of three. However, it is really possible to obtain an approximate set of values representing the initial xanthophyll content of these nine samples. These may readily be computed, on the assumption that the weights of the samples within each size-group stood in the same ratios to one another at the beginning of the experiment as they did at the end. Thus the sample lots W 101, 102 and 103 contained, respectively, 32.4%, 33.7% and 33.8% of the total mass (157.8 grams) at the end of the experiment, and the original mass (118.6 grams) may be apportioned in the same ratios. Each of these assumed original values for weight may then be multiplied by our assumed concentration (0.980) as before. Thus proceeding, we have a set of nine values for "absolute amounts of xanthophyll," which may be compared with the nine obtained at the end of the experiment. It is of significance that in every case the later figure exceeded the earlier one, the differences ranging from 4 to 23 per cent.

[8] Or possibly into other oxidation-products of carotene, indistinguishable from these by our qualitative chemical tests.

[9] Considerable time was devoted to ascertaining both the amount of this plankton in the piped sea-water, and the amount of its contained carotenoids, but under present circumstances, it does not seem worth while to discuss these.

[10] Confirmed spectroscopically by Dr. J. H. C. Smith and Mr. Harold W. Milner (Sumner and Fox, 1933).

[11] Such refined technique as that employed by Schertz (1923, 1925) and others is clearly indicated in the present case. Situations arise in which the identification of these substances by purely chemical technique is inadequate.

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SOME RARE AMPHIBIANS AND REPTILES OF THE UNITED STATES

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Read before the Academy, Monday, April 22, 1935

In our searches for live representatives of every species of the amphibians and reptiles of these United States, many interesting records of rare, recently described or little known forms have accumulated. Space permits but a few notes on some of the noteworthy species.

Amphibians.—1. *Ambystoma cingulatum* Cope.—This remains one of the rarest salamanders of the southeastern states. This gray-barred form occurs from Grahamville,[1] South Carolina, to Mobile, Alabama (J. Hurter). In recent times since 1914 (Deckert at Jacksonville, Fla.),

we know of no specimens being found. On the east edge of the Okefinokee Swamp, Georgia, we took one in a dry pine woods (June 15, 1922) and another near the edge of a cypress pond (July 23, 1922).

2. *Ambystoma mabeei* Bishop.—In 1928 Dr. S. C. Bishop[2] on one specimen from low grounds of the Black River near Dunn, N. C., described a new form, *Ambystoma mabeei*. No more have been reported since that time. In the spring of 1934 we took one (Feb. 24, 1934) 14 miles north of Wilmington, N. C., and about twenty-one more (Feb. 25, 1934) four miles east of the Edisto River on Route No. 17, Rantowles, S. C., and west of Charleston. This spring (1935) at this latter locality, Mr. H. G. M. Jopson found more. This new form may explain the former queer distributional records of supposed *Ambystoma texanum* (*microstonum*) east of the Alleghanies and only in North Carolina.

3. *Gyrinophilus porphyriticus duryi* (Weller).—Weller's[3] new form of the Purple salamander *Gyrinophilus porphyriticus duryi* (Weller) described in 1930 and known from northeastern Kentucky and southeastern Ohio, also occurs in West Virginia. We secured it at Polecat Run, Sisterville, West Virginia, June 22–27, 1931.

4. *Aenides aeneus* (Cope).—Cope[4] had one specimen from Nickajack Cave which is near the junction of Georgia, Alabama and Tennessee. Until Dunn's Plethodontidae appeared, no more than a dozen specimens had accumulated since 1881. Pope[5] July 20–28, 1927, Pine Mountain, Kentucky, made some good observations on this form. In June, 1931, in examining the West Virginia University Museum material, we found one *A. aeneus* labeled "Cheat River about 1920" (Cheat River usually meaning Cooper Rock country to the University staff). In July, 1931, Mr. M. Graham Netting independently sensed it as *A. aeneus*. Later that summer we went to Baileysville, W. Va., but knew not the secret of finding them in drought. At Athens, W. Va., July 21–25, 1931, Mr. P. S. Bibbee took us to Brush Creek Canyon where we accidentally found the cliff cracks their habitat. Later Mr. Netting[6] visited this locality. Still later he and Mr. N. Richmond began a search of Cooper's Rock where they found several. The first specimen we ever saw was taken June 15–25, 1909, by Professor J. C. Bradley at Tallulah Falls, Georgia. Many years later Oct. 18, 1926, at this place Doctor S. C. Bishop found it on a boulder under some wood. It is no longer uncommon now we know its habitat.

5. *Leptodactylus albilabris* (Günther).—In 1931 near Los Olmos bridge, Rio Grande City, Texas, Doctor E. H. Taylor[7] and J. S. Wright took one specimen of this species. It occurs from Vera Cruz to Nicaragua; also in Porto Rico and the Virgin Islands. On May 2, 1934, Mr. Stanley Mulaik of Edinburg, Texas, described a frog which sounded like sea lions and he gave me two specimens of this species. They are the second and third specimens of the White-lipped Frog from the United States.

6. *Rana heckscheri* Wright.—Since we[8] described this form in 1924 this species has been found sporadically from South Carolina to Biloxi, Mississippi. Several have been found in Florida and, imagine my surprise in finding that E. Ross Allen at Silver Springs, Florida, considers it one of his main local reliances as food for his reptile house.

Reptiles.—7. *Crotaphytus reticulatus* Baird.—From Baird's[9] first description in 1858 to 1898 only four specimens accumulated for Cope[12] to pronounce it still rare. The Biological Survey of Texas[10] published in 1905, reports that "William Lloyd collected a specimen of this rare and apparently very locally distributed lizard at Rio Grande City, Texas, May 28, 1891." In 1925 (June 12–16) we saw them from Rio Grande City to Roma, Texas, and also in 1934, in which year Mr. Stanley Mulaik of Edinburg, Texas, took specimens.

8. *Sceloporus disparilis* Stejneger.—In 1905 Doctor Stejneger[10] identified "five specimens collected by Mr. William Lloyd at Lomita Ranch, six miles north of Hidalgo, Texas," as *Sceloporus dispar*, Baird and Girard. Later, in 1916, Doctor Stejneger[11] described these five and two specimens from Duval County, Texas, and Nuevo Leon, as a new species, *S. disparilis*. So far as we know, no one has recorded it since the three lots were taken. On May 2, 1934, while visiting Mr. Stanley Mulaik's school in Edinburg, Texas, we saw a recently hatched young of this species, and later, June 14, 1934, we received a live adult female. In 1925 at La Lomita we did not find it because of a drought.

9. *Sceloporus torquatus cyanogenys* Cope.—In 1898 Cope[12] writes "*S. t. cyanogenys* is found so near the boundary that it is not unlikely to be found within our limits. Indeed I am not sure that I did not see this animal on the rocky banks of the Rio Grande at Laredo, Texas, in 1885." In 1925 from June 9–15 we found this form from Zapata to Roma, Texas. At the latter place we took a fine specimen on June 15. In 1934 we found several around Rio Grande City where E. H. Taylor[13] captured it in 1930. Doctor Taylor was the first to announce its presence in the United States.

10. *Neoseps reynoldsii* Stejneger.—This elongate burrowing skink with one toe on the fore foot and two toes on the hind foot has eluded most collectors since Doctor Stejneger[14] described the unique specimen from Fruitland Park. Thereafter, one man, Mr. Norman Wood, in Auburndale, Florida, collected them in considerable number, while no one else ever found them, or very rarely. In 1934 (March 30) Mr. O. C. Van Hyning at Eustis, Florida, when directing a large group of CCC workers in making an airport, found that in the first time over they went down three inches and found mainly *Eumeces egregius*. The next time, they went down to nine inches and revealed mainly *Rhineura floridana* and *Neoseps reynoldsii*.

11. *Coluber constrictor flaviventris* (Say).—In 1895 Cope[12] described

two new forms of *Zamenis* from the lower Rio Grande valley: one as *Z. stejnegerianus* from one specimen from Cameron County; and another as *Z. conirostris* from one specimen from Matamoras. Brown[15] in 1901 considered the first as synonymous with *Zamenis constrictor flaviventris*. In 1928 Doctor Ortenburger[16] in his monograph on the racers following Brown considers the two forms *Coluber c. flaviventris* but shows in his range map no *Coluber c. flaviventris* below the latitude of Victoria, Texas. In 1934 I saw at Edinburg, Texas, one specimen which more or less fits the composite description of Cope's 1895 forms. Later, in 1934, we received another specimen of the same form from Edinburg. We suspect these two species will become one, *Coluber stejnegerianus*. Eventually it may be considered *C. c. flaviventris*, but I believe more work and material are needed before a conservative accepted determination is possible.

12. *Elaphe bairdii* (Yarrow).—In 1880 Yarrow[17] described *Elaphe bairdii* from Ft. Davis, Texas. Until a few years ago no other specimens were known. Doctor G. S. Myers and Mr. G. M. Kranzthor secured one in the Cherry Canyon, Ft. Davis Mountains, on May 20, 1929. He also found one in the Sul Ross Teachers College collection at Alpine, Texas. In 1917 and 1925 we saw it not, but in the Chisos Mts., May 21, 1934, just as we were breaking camp in Green Gulch, we picked up the fourth specimen known.

13. *Elaphe rosacea* (Cope).—In 1888 Cope[18] described this form from one fresh alcoholic specimen taken at Key West, and mentioned four indistinct longitudinal bands. In 1920 Doctor Thomas Barbour[19] reported on a beautiful live *Elaphe rosacea* taken on Big Pine Key. Like Cope, he noted its intermediate position between *E. guttata* and *E. quadrivittata*. In 1933 Mr. Maurice Brady[20] reported on the third specimen found and, ostensibly, it came from Little Pine Key. Last spring (March 18, 1934) on our lone day at Matacumbe Key, we went out at night for *Hyla septentrionalis*, and fortunately stumbled on this fourth specimen of *Elaphe rosacea*, but it has no suggestion of four longitudinal stripes. Mr. A. Pfleuger, who captured Mr. Brady's specimen, said he had two more which were lost. He saw my specimen in life and considered it the same as Brady's specimen and the two he lost. Is this form moving northward as *Hyla septentrionalis E. ricordii* and others are doing? On March 14, 1935, we received another specimen from Mr. H. G. M. Jopson at Key West.

14. *Elaphe subocularis* (Brown).—E. Meyenburg collected snakes in Ft. Davis Mts., for Mr. Arthur E. Brown[21] of the Philadelphia Zoo. Among his finds was a new form described in 1901. Until Colonel M. L. Crimmins found a specimen[22] at Mt. Franklin, El Paso County, its range was held to be solely Ft. Davis Mts. It is yet held to be a very rare form. After we had left the Big Bend country and Chisos Mt. country, Mr. Thomas

Miller brought me at Alpine a beautiful specimen from San Vicente, Texas, very near the tip of the Big Bend of the Rio Grande River.

15. *Lampropeltis alterna* (Brown).—After A. E. Brown published *Elaphe subocularis* above, he sent a copy of his paper on *E. subocularis* to the collector, E. Meyenburg. This last year Mrs. Ellen Schulz Quillen of San Antonio gave me a number of papers she had bought in San Antonio several hundred miles from Meyenburg's operations. Among them was this separate to Meyenburg. Lo and behold! on the back cover was a photograph from life of Meyenburg's latest find, *Lampropeltis alterna*.[23] Only one specimen was ever found and no photos of it were known when Doctor Blanchard[24] published his thesis.

16. *Lampropeltis getulus brooksii* Barbour.—In 1919 Doctor Thomas Barbour[25] described this tropical Floridan race wherein no light dorsal crossband appears, as in all other *L. getulus* subspecies. I have had several specimens which closely approached this form but no perfect duplicate of it. At Pinecrest, Florida, in 1934 (March), Mr. M. B. Bishop and his associates from New Haven took three *L. getulus floridanus* and one *Lampropeltis g. brooksii*. We saw the four. As yet, we are at a loss to understand this form. Several live specimens which various collectors have called this subspecies have certainly appeared to be *L. getulus floridanus*.

17. *Stilosoma extenuatum* Brown.—For many years after its description[26] until 1925 or 1928 this form was considered very rare. In recent years a few have been found. In 1934 Mr. E. Ross Allen of Silver Springs, Florida, told me he had taken 15–20 of them. They feed on other snakes and constrict them.

18. *Ficimia streckeri* Taylor.—In 1931 Doctor E. H. Taylor[27] described a new species of *Ficimia* as *F. streckeri*. He secured it three miles east of Rio Grande City, Texas. I received another specimen of this form from Brownsville, Texas. It, therefore, ranges from Rio Grande City to Brownsville, Texas.

19. *Coniophanes imperialis* (Baird).—In 1898 Cope[12] apparently had just the one alcoholic specimen from Captain Van Vliet's collections. On October 15, 1929, we received one specimen from Brownsville, Texas. On April 25, 1934, Mr. A. J. Kirn of Somerset, Texas, showed me a snake he took near Rebb's Palm Grove, Brownsville, Texas. It proved to be of this species. On May 2, 1934, we visited Mr. and Mrs. Irby Davis of Harlingen to see if a snake they had was alive. That morning Mrs. Davis had tried to etherize it, but did not succeed, and in the afternoon of the same day we saw it—our first one alive. Doubtless this form is not uncommon in the Rio Grande Valley.

20. *Kinosternon bauri palmarum* Stejneger.—On December 4, 1925, Doctor L. Stejneger[28] described this translucent mud turtle wherein the dermal carapace sutures show through the shell. We have specimens

of this form from the southern tip of Florida. In 1934, with Mr. and Mrs. H. G. M. Jopson, we saw another specimen at the Miami Aquarium. But the striking discovery was to find that some of the students around Gainesville, Florida, had also taken two or three specimens at that place.

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STUDIES OF PROTECTIVE COLOR CHANGE. III. EXPERIMENTS WITH FISHES BOTH AS PREDATORS AND PREY

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In an earlier issue of these PROCEEDINGS[1] I described some experiments designed to test the protective value of concealing coloration to organisms equipped with the mechanism for adjusting their color-scheme to that of the background. Small fishes (*Gambusia patruelis*) were chosen for this purpose, a fish-eating bird (penguin) being selected as the predator. These

results afforded unmistakable evidence that fishes which are not well adjusted to the color (more strictly the *shade*) of their background are captured in preference to those which are better adjusted. The numbers of fishes used were considerable and the differences in survival rates were great.

Since conducting these earlier experiments with penguins another fish-eating bird, this time a wader, was used as the predator, while the former birds were again employed in a supplementary series of experiments. These results, just published,[2] furnish additional evidence of the protection afforded by relative inconspicuousness when fishes are preyed upon by birds. This selective predation on the part of birds was still evident, even after the initial difference between the two lots of fishes had been greatly reduced by sojourn upon a common background. It was also demonstrated that the differential mortality of the two lots did not result from any difference in their ability to outswim their pursuers.

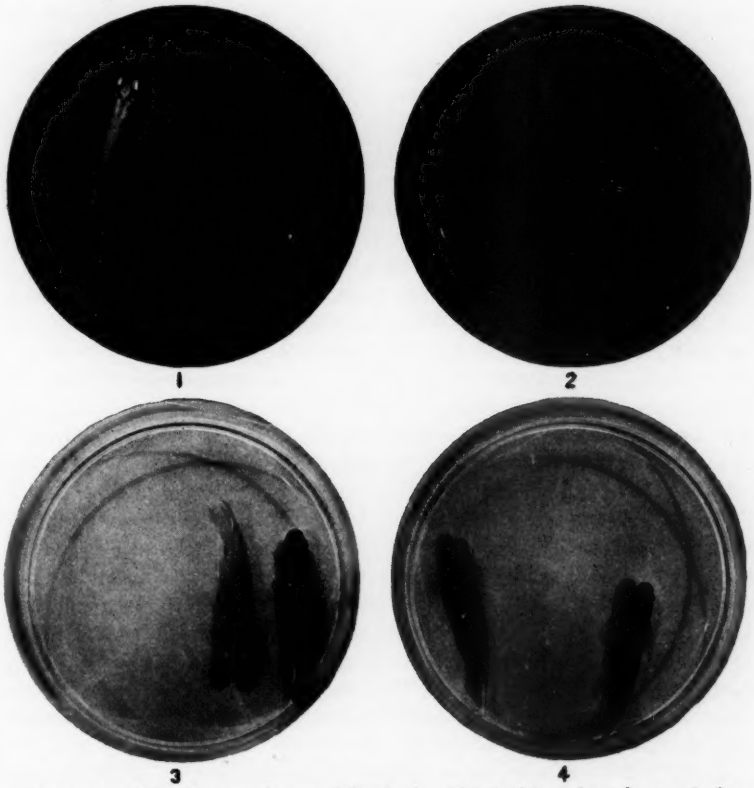
At the time of performing these experiments, I realized that smaller species of fish are probably preyed upon to a greater extent by larger fishes than by birds. Any test of the effectiveness of the mechanism for concealment must therefore take into account this more numerous class of predators.

A few preliminary experiments with the large-mouthed black-bass (*Micropterus salmoides*) were undertaken. But several features of my procedure at this time rendered the results of these experiments worthless, and this test was therefore deferred to a more favorable opportunity. Later, two specimens of a supposedly voracious Chinese species (*Channa fasciata*) were secured, and the attempt renewed. But these fishes were not very successful in catching the *Gambusia*. It is worth recording, however, that about twice as many "whites" as "blacks" were devoured in the course of a few trials in the black tanks.

The real test with a predaceous fish was made with the blue-green sunfish (*Apomotis cyanellus*). Twenty-five specimens of this species were caught in a pool which had been stocked some years ago.[3] These ranged from 7 to 14 cm. in length, but it is probable that at least half of the specimens were too small to devour the *Gambusia*. The latter, for the most part, ranged in length from 3 to 5 centimeters. Before the commencement of the experiment they had been kept for 72 days in cement tanks painted black and white, respectively. As stated in my earlier accounts these two lots differed glaringly when first compared upon a common background. The difference decreased perceptibly within a minute or two, however, and was greatly reduced during the first hour or less (Figs. 2, 4). But some degree of difference commonly remained discernible to the human eye for several days. At no time in the course of the experiments here reported was any real difficulty encountered by me in distin-

guishing fishes having these contrasting histories. Commonly recognition was immediate.

For the feeding tests a cement tank was used, having a bottom area of 187 × 55 centimeters. Running fresh water was passed through this tank, and kept, in most of the experiments, at a level of 30 cm. The walls and bottom of the tank were painted a pale yellowish gray, approximating Ridgway's "light olive gray" or "light grayish olive." For those experiments in which a black background was required, the interior of the tank was not itself painted, but a tight-fitting, black wooden box was inserted as a lining.



Figures 1 and 2.—Two specimens of *Gambusia* which had been kept for nearly four months in black and white tanks, respectively, then photographed upon black background. Figure 1, after 10 seconds; figure 2, after 2 hours.

Figures 3 and 4.—Two specimens of identical history, photographed upon pale gray background, very similar to that of tank used in experiments. Figure 3, after 35 seconds; figure 4, after 10 minutes.

(Photographs by Nelson A. Wells and the writer.)

The sunfish were kept in this experimental tank[4] and the *Gambusia* were introduced as occasion demanded. Commonly, 25 each of the "blacks" and "whites" were poured in simultaneously, but the procedure was varied to suit the requirements of the experiment. The food-fishes were left in for a period of from one minute to several hours before being removed and counted. Since most of the feeding occurred within the first few minutes, this period, in a large majority of cases, was less than fifteen minutes.

The data derived from these 29 experiments are recorded in table 1. The more essential results of these studies may be stated briefly as follows:

(1) In four experiments with the black tank, both "white" and "black" *Gambusia* were transferred directly from their customary backgrounds to the experimental tank. Thirty-seven of the 200 food-fishes were eaten, i.e., 18.5 per cent. Of these 37 fishes, 8 (22%) were "blacks," 29 (78%) being "whites."

(2) In four other experiments with the black tank, the "white" fishes were kept for a preliminary period of varying length in a black container before being transferred (along with the "blacks") to the experimental tank. This was in order that the differences in appearance between the two lots might be considerably reduced. Such treatment, for periods up to two hours, seems to have had little effect upon the outcome. In two experiments thirty-four fishes were eaten, of which 8 (24%) were blacks and 26 (76%) were whites. When the "whites" had been subjected to darkening for 23 hours, on the other hand, 9 "blacks" and 4 "whites" were eaten. After treatment for two days the numbers were 6 and 4, respectively, the initial number of each in this last case being only 13. This heavier mortality of the black fishes, under these conditions, is hard to account for. It may be purely accidental.

(3) In nine experiments with the pale gray tank, both "black" and "white" *Gambusia* were transferred, in equal numbers, directly to this tank without preliminary treatment of the "blacks." Of the 430 fishes used, 208 (48%) were eaten. This much heavier consumption of the *Gambusia* in the pale tank cannot probably be regarded as significant. It depends partly on the fact that the sunfishes had, by this time, become more fully accustomed to eating the *Gambusia* in the presence of observers, partly upon my selection, in the later experiments, of the smallest food-fishes available. (Many of the others proved to be too large to be readily caught or swallowed by the sunfish.)

Of the 208 which were eaten in these nine experiments, 137 (66%) were "blacks," 71 (34%) being "whites." These figures imply a lower degree of selectivity on the part of the sunfish in the pale-tank experiments than in those with the black one. It is of interest that this same relation held in the experiments with penguins, and, indeed, that the proportions of "black" and "white" fishes eaten in the two tanks agree fairly closely

TABLE 1

NUMBERS OF "BLACK" AND "WHITE" *Gambusia* DEVOURED BY *Apomotis* UNDER VARIOUS EXPERIMENTAL CONDITIONS

	NO. OF EXPERIMENT	DATE 1935	NO. OF "BLACKS" OFFERED	NO. OF "WHITES" OFFERED	PREVIOUS SOJOURN ON COMMON GROUND	DURATION OF TEST	NUMBERS EATEN "BLACKS"	NUMBERS EATEN "WHITES"	PERCENTAGES "BLACKS"	PERCENTAGES "WHITES"
Black tank	1	2/13	25	25	0	1 hr. 45 min.	1	5		
	2	14	25	25	0	2 hrs.	2	7	22	78
	3	15	25	25	0	5 hrs.	3	5		
	4	18	25	25	0	15 min.	2	12		
	5	20	25	25	16 min.	2 hr. 40 min.	4	9	24	76
	6	25	25	25	2 hrs.	20 min.	4	17		
	7	27	25	25	23 hrs.	4 hrs. 30 min.	9	12	48	52
	8	28	13	13	48 hrs. (±)	35 min.	6	4		
Gray tank	9	3/6	25	25	0	10 min.	6	5		
	10	7	25	25	0	5 min.	17	12		
	11	9	25	25	0	3 min.	18	11		
	12	11	25	25	0	2 min.	16	10		
	14	13	25	25	0	1¼ min.	24	10	66	34
	18	18	15	15	0	66 sec.	15	3		
	19	18	25	25	0	3 min.	14	11		
	23	22	25	25	0	30 min.	10	2		
	28	4/1	25	25	0	3 min.	17	7		
	16	3/15	16	32	0	1 min.	13	8	67	33
	24	23	16	32	0	3 min.	9	3		
	13	12	25	25	1 hr. 20 min.	5 min.	10	14		
	15	14	25	25	30 min.	2 min.	14	12		
	17	16	25	25	15 min.	2 min.	9	10	49	51
	25	27	25	25	10 min.	3 min.	5	0		
	26	28	20	20	10 min.	12 min.	4	6		
	27	30	25	25	10 min.	3 min.	8	10		
	20	20	15 ur. 15 con.			1½ min.	15 ur. 3 con.			
	21	20		25 adr. 25 con.		2 min.		25 adr. 2 con.		
	22	21		25 adr. 25 con.		?		22 adr. 2 con.		
	29	4/2	25	25 adr.		2 min.	5	20		

in the two cases. It is likewise a result which an observer might readily have forecast. It is much easier for the human eye to see one of the "white" fishes in the pale tank than to see one of the "black" fishes in the black tank.[5]

(4) Two of the experiments (9 and 19), conducted in the late afternoon when the light was dim, seemed to indicate that selection on the basis of visibility was less intense at such times.[6] But the numbers are few.

(5) In a number of the foregoing experiments, in which equal numbers of the "black" and "white" *Gambusia* were offered to the sunfishes, all or most of the "blacks" were devoured, while a majority of the "whites" remained uneaten. This means, of course, that in the later stages of such an experiment the "whites" greatly outnumbered the "blacks" in the tank. Despite this fact, the sunfish continued to pursue the latter and largely to ignore the former.

As a further test of this tendency, two experiments were performed (numbers 16 and 24) in which twice as many "whites" as "blacks" were introduced at the outset. It is of interest that of the 33 fishes eaten 67 per cent were "blacks" and 33 per cent "whites." These percentages, it will be recalled, are almost precisely the same as those obtained when equal numbers of "blacks" and "whites" were offered. Furthermore, if we consider for each lot the proportion of the number offered which was eaten, we find this to be 69 per cent for the "blacks" and 17 per cent for the "whites." Thus "availability," in the spatial sense in which this word seems to be chiefly employed by McAtee, has had little to do, in the present case, with the choice made by the predators.

(6) As in the case of the black tank, a number of experiments were performed with the gray one, in which the food-fishes offered had been subjected to treatment which greatly reduced their initial differences. The treatment in the present case consisted in keeping the "blacks" upon a white background for a varying period before they were poured (in company with the "whites") into the experimental tank. This period ranged from 10 to 80 minutes. In the present experiments the convergence proved to be sufficiently great within even the ten-minute period to completely nullify the difference in survival rate. Of the 290 *Gambusia* offered to the sunfishes in the course of six experiments, 102 (35%) were eaten. The proportions of "blacks" and "whites" were nearly identical (49% and 51%, respectively).

This result may seem surprising, in view of the evident difference to the human eye between the two sets of fishes, even after keeping the "blacks" on a pale background for ten minutes (Fig. 4). The result is likewise quite different from that obtained in the later penguin experiments,[7] in which a marked degree of choice was still evident after keeping both sets of fishes in the pale tank for 20 to 27 hours.

However, the penguin experiments differed from those with sunfishes in certain important respects. First, the entire lot of *Gambusia* employed in the latter experiments had been kept for more than a month in a nearly white tank before transferring them to white and black tanks, preparatory to the experiments here described. Because of this history, it seems likely that these later "blacks" would more readily pale when transferred to a pale background.

In the second place, the pale tank used in the sunfish experiments was somewhat darker than that used in the experiments with birds. The fact that, after a period of "bleaching," an originally black *Gambusia* still appeared distinctly darker than this background, to the human eye, is not decisive as to its appearance to the sunfish. The human observer views these fishes from above, or nearly so. The piscine predator views them from the side or even from below. It may well be that, against the background here used, the "whites" and the (bleached) "blacks" were equally visible (or invisible) to their pursuers.

(7) Four experiments (20, 21, 22, 29) were performed for the purpose of determining the influence of motion versus quiescence upon the chances of survival of these little fishes. Numerous authors (e.g., Young[8] and Cuénot[9]) have pointed out the importance of quiescence in protecting helpless organisms from predators. Cuénot describes an experiment of his own in which certain shrimps proved to be safe, regardless of their color, so long as they remained motionless, though they were promptly seized by fishes as soon as they moved. My experiments dealing with this subject make it plain that the situation is altogether different in the case of *Gambusia* and *Apomotis*.

In the first of these experiments (No. 20), 15 black *Gambusia* were subjected to a 1% solution of urethane until they were completely narcotized. This drug has the effect of anesthetizing the fish and likewise of maintaining it in a condition of maximum blackness. These 15 fishes, after rinsing, were introduced along with another 15 "blacks" into the gray experimental tank. The *Gambusia* were poured in at one end while the sunfishes were at first kept at a distance by the insertion of a wooden partition. When the latter was removed, the sunfishes promptly attacked the anesthetized *Gambusia* and consumed the entire lot within one and a half minutes, at which time only three of the active ones had been taken.

In a second experiment, 25 "whites" were subjected to a 1:2000 solution of adrenalin. This drug, likewise, narcotizes the fish but its effect upon the chromatophores is opposite to that of urethane. The fishes retain their high degree of pallor. In this experiment, the sunfishes devoured all of the 25 anesthetized *Gambusia* within two minutes, during which time only two of the controls had been eaten.

Another experiment was conducted with "white" fishes subjected to

adrenalin. In this case, the water was diminished in depth from 30 centimeters to 13. In the two experiments first described, in which deeper water was used, the anesthetized fishes mostly sank to the bottom while the controls swam actively near the surface. The reduction of the water level was for the purpose of bringing the two lots into closer association. The result of the feeding test was, nevertheless, almost identical with the others. Twenty-two of the anesthetized fishes and only two of the controls were devoured during the period of the experiment.

It is plain that active locomotion, far from exposing these food-fishes to greater hazard, was of vital importance in enabling them to escape their pursuers. In order to evaluate the relative efficacy of these two methods of protection (concealment and flight) in the present circumstances, one decidedly inadequate experiment was performed (No. 29). Twenty-five "white" *Gambusia* were subjected to adrenalin, while 25 "blacks" were left untreated. In the course of two minutes 20 of the motionless "whites" were devoured and only 5 of the active "blacks." One cannot be certain, however, that the results would have been the same had the *Gambusia* used been smaller. By this time most of the specimens were too large to serve well as living prey for the sunfishes.

The foregoing experiments, in the aggregate, make it plain, first, that relative conspicuousness in a given environment, far more than availability in the spatial sense, determines the survival or non-survival of small fishes under attack by piscine predators; and, second, that total quiescence is a handicap rather than an advantage to such fishes in escaping capture. These statements are subject, of course, to the customary qualification: "at least for the species used, and under the conditions of these experiments." But the species used are believed to be representative, each of a vast array of species with similar habits; while the conditions of these experiments are not believed to be such as to enhance the selectivity in feeding, but rather to diminish it. For the chances of capturing an inconspicuous fish are probably greater in an experimental tank than in a large natural body of water.

Granting these facts, the utility of the chromatophore mechanism by which these color changes are effected is hardly open to doubt. And since there exist wide differences in the perfection of this mechanism, between individuals as well as between species, it seems probable that natural selection has played an important part in its evolution.

[1] *Proc. Nat. Acad. Sci.*, **20**, 559–564, October (1934).

[2] *Am. Nat.*, **69**, 245–266 (1935).

[3] This species is not native to California.

[4] It is of interest that the sunfish itself undergoes color changes nearly or quite as marked as those undergone by the *Gambusia*. The sunfish, especially when young, is

doubtless preyed upon by larger species. Perhaps, also, we have to do here with the concealment of the predator from prospective prey.

[5] This relation did not hold for the night-heron, however. In the case of this bird, the intensity of selection was somewhat greater in the pale tank.

[6] In the second of these experiments, the curtains were drawn and the light so dim that newspaper print could be read only with difficulty.

[7] See footnote 2

[8] *Jour. Exper. Zoöl.*, **20**, 457–507 (1916).

[9] *Annal. Sci. Naturel.* (Ser. Bot. et Zool.), T. 10, 123–150 (1927).

SUMMATION OF MULTIPLE FOURIER SERIES BY SPHERICAL MEANS

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Let $x = (x_1, \ldots, x_k)$ be a point in the k-dimensional Euclidean space, and $f(x)$ a function of the Lebesgue class L having the period 2π in each variable, and let

$$f(x) \sim \sum a_{n_1 \ldots n_k} e^{i(n_1 x_1 + \ldots + n_k x_k)} \tag{1}$$

be its Fourier series. If $\varphi(t)$ is a fixed function in $0 \leqq t < \infty$ for which $\varphi(0) = 1$, then, under unessential restrictions about the behavior of $\varphi(t)$ at ∞, we may form the "partial sums"

$$S_R(x) = \sum \varphi\left(\frac{v}{R}\right) a_{n_1 \ldots n_k} e^{i(n_1 x_1 + \ldots + n_k x_k)}, \qquad v^2 = n_1^2 + \ldots + n_k^2. \tag{2}$$

and consider conditions under which $S_R(x) \longrightarrow f(x)$ as $R \longrightarrow \infty$. For instance, if $\varphi(t) = e^{-t}$ we have (spherical) Abel-Poisson summation, and for

$$\varphi(t) = \begin{cases} (1-t)^\alpha & 0 \leqq t \leqq 1 \\ 0 & 1 < t \end{cases} \tag{3}$$

we have the important case of summation (R, α) (summation by spherical Riesz's means of order α).

The spherical partial sums (2) have a great advantage over the usual rectangular ones. In the case of rectangular partial sums it is necessary (and natural) to consider, for $k \geqq 2$, the behavior of the given function $f(x)$ in a "cross-neighborhood" of the given point x. This complicating feature is eliminated entirely in the case of spherical partial sums (2), as is evident from the formula.

$$S_R(x) = \int_0^\infty f_x(t)\, H(t, R)\, dt^1 \tag{4}$$

where $H(t,R) = \frac{1}{t}\int_0^\infty \varphi\left(\frac{u}{tR}\right) u^{l+1} J_l(u), l = \frac{k-2}{2}$, $J_l(u)$ is the Bessel function of order l; and $f_x(t)$ is the $(k-1)$-dimensional spherical mean of the function $f(x)$ around the point x:

$$f_x(t) = \frac{\Gamma\left(\frac{k}{2}\right)}{2\pi^{k/2}} \int f(x_1 + t\xi_1, \ldots, x_k + t\xi_k) d\xi_1 \ldots d\xi_k.$$

$$\xi_1^2 + \ldots + \xi_k^2 = 1.$$

Thus, irrespective of whether the summation function $\varphi(t)$ is a "good" or "bad" one, what decides the convergence of the partial sums (2) are only the spherical averages of the function $f(x)$ around the one point x and nothing else. Also, the formula (4) and the convergence criteria based on this formula, are by no means restricted to periodic functions. In fact, the function $f(x)$ may be the most general almost periodic function in k variables and this implies that the numbers $n_1, \ldots, n_k$ in (1) and (2) may be any real numbers (and not only integers). It is true that the more delicate summation criteria do not hold for almost periodic functions in general, but it appears that the pure periodicity is required only in this way that for integers $n_1, \ldots, n_k$ the distribution of the points $(n_1, \ldots, n_k)$ in the k-dimensional space is not too dense. In other words, the more delicate criteria appear as "gap-theorems" for functions $f(x)$ of an unrestricted character of almost periodicity. For $k = 1$ this viewpoint has been previously justified by the author.[2]

A detailed treatment of the subject will be given in a paper which will appear in the Transactions of the American Mathematical Society. For the present we shall state a few of the theorems.

1. If $\varphi(t)$ has more than $\frac{k+1}{2}$ derivatives and is sufficiently small at ∞, then $S_R(x) \longrightarrow f_0$, provided that $\int_0^t |f(t) - f_0|\, dt = 0(t)$ for $t \longrightarrow 0$.

2. For the summation function (3) the same statement holds as soon as $\alpha > \frac{k-1}{2}$.

3. If $\alpha = \frac{k-1}{2}$ one of the following two additional restrictions is sufficient: (i) the function $f_x(t)$ has the Fourier behavior as $t \longrightarrow 0$, and the number of those terms in (1) for which $R \leqq \nu < R + 1$ is $0(R^{\frac{k-2}{2}})$ as R

$\longrightarrow \infty$; (ii) for some $p > 0$, the number of terms for which $R \leqq \nu < R + 1$ is $0(R^p)$ and the number of terms for which $R \leqq \nu < R + R^{-p}$ is $0\left(R^{\frac{(p+1)(k-1)}{2}}\right)$, and

$$\int_\delta^{\delta^p} \frac{|f(t) - f(t+\delta)|}{t} dt \longrightarrow 0 \text{ as } \delta \longrightarrow 0.$$

4. The case $\alpha = \frac{k-1}{2} - \beta$, $0 < \beta < 1$ requires a uniform Lipschitz condition of order β in conjunction with a suitable restriction on the density of the exponents.

[1] For multiple Fourier integrals in the place of Fourier series this formula has been previously established and made use of in the paper: S. Bochner, "Ein Konvergenzsatz fur mehrvariablige Fouriersche Integrale," *Math. Zeit.*, **34**, 440–447 (1931); see also A. C. Berry, "The Fourier Transform Theorem," *Jour. Math. Phys. Mass. Inst. Technology*, **8**, 106–118 (1929).

[2] See Bochner, S., "Properties of Fourier Series of Almost Periodic Functions," *Proc. Lond. Math. Soc.*, **26**, 433–452 (1925).

THE AUTOMORPHISMS OF CAYLEY'S NON-ASSOCIATIVE ALGEBRA

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This paper is concerned with a particular type of vector-algebra[1] over the field of all real numbers. We have a vectorial product $\mathfrak{a} \times \mathfrak{b} = -\mathfrak{b} \times \mathfrak{a}$, zero only if $\mathfrak{a}$ and $\mathfrak{b}$ are linearly dependent; and a scalar product $\mathfrak{a} \circ \mathfrak{b} = \mathfrak{b} \circ \mathfrak{a}$, which is a bilinear real function of the two vectors $\mathfrak{a}$, $\mathfrak{b}$; $\mathfrak{a} \circ \mathfrak{a}$ is a positive definite form.

These two multiplications are connected by

(1) The determinant law $(\mathfrak{a} \times \mathfrak{b}) \circ \mathfrak{x} = \mathfrak{a} \circ (\mathfrak{b} \times \mathfrak{x})$,

(2) The (weak) identity of Lagrange,

$$(\mathfrak{a} \times \mathfrak{b}) \times \mathfrak{b} = \mathfrak{b}(\mathfrak{a} \circ \mathfrak{b}) - \mathfrak{a}(\mathfrak{b} \circ \mathfrak{b}).$$

Applying these rules one proves:

(1) If $\mathfrak{a}$ and $\mathfrak{b}$ are linearly independent, then $\mathfrak{a}$, $\mathfrak{b}$, $\mathfrak{a} \times \mathfrak{b}$ are a basis of a vector algebra of the order three, which is isomorphic to the algebra defined by the ordinary three-dimensional vector calculus.

(2) If furthermore $\mathfrak{x}$ is independent from $\mathfrak{a}$, $\mathfrak{b}$, $\mathfrak{a} \times \mathfrak{b}$, the seven vectors

$$\mathfrak{a}, \mathfrak{b}, \mathfrak{k}, \mathfrak{a} \times \mathfrak{b}, \mathfrak{b} \times \mathfrak{k}, \mathfrak{k} \times (\mathfrak{a} \times \mathfrak{b}), \mathfrak{a} \times \mathfrak{k}$$

are a basis of the *entire* vector algebra, which therefore has the order 7.

(3) If moreover these three vectors are orthonormal with respect to scalar products—we shall denote them in this case i_1, i_2, i_3—then we get a system of seven *orthonormal* vectors $i_1 \ldots i_7$. Their multiplication is given by the formulas:

$$i_1 \times i_2 = -\, i_2 \times i_1 = i_4$$
$$i_\nu \times i_{\nu+1} = i_{\nu+3}$$

where the indices have to be taken mod. 7. Let us call a set of vectors of that kind a "Cayley basis" of our system of "Cayley vectors."

A Cayley basis is determined by the first three vectors, which have to be orthonormal, with "determinant" $(i_1 \times i_2)\ 0\ i_3$ zero.

This is very useful for the study of the automorphisms. An automorphism is completely defined by the images of the first three vectors, e.g., there is an automorphism σ such that

$$\sigma(i_1) = i_1$$

$$\sigma(i_2) = \cos\varphi_1 i_2 + \sin\varphi_1 i_4 \quad \text{(rotating } i_2 \text{ in the plane } (i_2, i_4) \text{ through } \varphi_1)$$
$$\sigma(i_3) = \cos\varphi_2 i_3 + \sin\varphi_2 i_7 \quad \text{(rotating } i_3 \text{ in the plane } (i_3, i_7) \text{ through } \varphi_2)$$

One verifies very easily that these three vectors satisfy the conditions and hence generate a Cayley basis. Moreover: the three planes (i_2, i_4) (i_3, i_7) (i_5, i_6) are invariant planes and undergo rotations of angles φ_1, φ_2, φ_3, respectively. The third angle has of course to be determined by φ_1 and φ_2. As a matter of fact, this is done by the simple relation

$$\varphi_1 + \varphi_2 + \varphi_3 \equiv 0\ (2\pi).$$

Our first main statement is the converse: *Each automorphism belongs in this manner to at least one Cayley basis.* The angles φ_ν are class invariants; two automorphisms with equal angles are conjugates in the group of all automorphisms.

Cayley vectors generate the famous system of Cayley numbers in the same way that the ordinary three dimensional vectors generate quaternions: Take symbols $\alpha + \mathfrak{a}$, where α is a real number, $\mathfrak{a}$ a vector, with the multiplication rule

$$(\alpha + \mathfrak{a})(\beta + \mathfrak{b}) = \alpha\beta - \mathfrak{a} \circ \mathfrak{b} + \mathfrak{a}\beta + \alpha\mathfrak{b} + \mathfrak{a} \times \mathfrak{b}.^1$$

This is a non-associative division algebra over the field of all real numbers; it may be characterized completely in the following manner: One Cayley number behaves exactly like a complex number, such that we can speak of real part, trace, norm, value of a rational function, equations satisfied, roots of unity. *Two* Cayley numbers behave like two quater-

nions, such that, e.g., $a^{-1} \times a$, the transform, is defined. We consider vectors as Cayley numbers with real part zero.

The automorphisms of the quaternions are well known; they are, so far as the vectors are concerned, the group of all orthogonal transformations with determinant one in three dimensions; algebraically, they are all *transformations* $a^{-1} \times a$. The automorphisms of the Cayley systems form a well-known group of fourteen parameters;[2,3] but in general they are not generated by transformations. On the contrary, there is a theorem of *Brandt*, which tells us that such a transformation $a^{-1} \times a$ is an automorphism if and only if the transformer is proportional to a sixth root of unity.

This can be proved very conveniently by our theory of automorphisms, in particular the relation $\varphi_1 + \varphi_2 + \varphi_3 = 0$, and the characterizing properties of the Cayley numbers. But there is also a more direct proof, which yields a slight generalization of Brandt's theorem: The difference

$$a^{-1}(xy)a - a^{-1} \times a.a^{-1}ya$$

is zero if and only if either, (1) a is a Brandt transformer, or (2) a, b and x lie in an associative subsystem. One has only to calculate the difference in terms of the real and vectorial parts of the numbers involved.

This statement may be applied to *double* transformations $b^{-1}(a^{-1} \times a)b$. They are automorphisms if either (1) a and b both lie in a commutative subsystem *and* have a product which is a Brandt transformer, or (2) a and b are both Brandt transformers. The theory of triple transformations is not finished yet. If in $\sigma(x) = c^{-1}(b^{-1}(a^{-1} \times a)b)c$ the three transformers are not all Brandt transformers, they have to be elements of an associative subsystem. I believe that they are in this case proportional to at most eighth roots of unity. I believe that each automorphism is the product of three Brandt transformations; I can prove that each automorphism may be generated by six transformations with fourth roots of unity

$$i_2^{-1}(j_2^{-1}(i_3^{-1}j_3^{-1}(i_5^{-1}(j_5^{-1} \times j_5)i_5)j_3)i_3)j_2)i_2.$$

Here the i_ν are taken out of an arbitrary Cayley basis belonging to σ, and the j_ν are the corresponding vectors of a Cayley basis, which is associated with σ and the basis i_ν in a unique manner.

I expect many generalizations: to Cayley numbers with arbitrary or with algebraic coefficient fields as well as to the hypercomplex non-associative systems which generalize Cayley's algebra of order eight.[1] The automorphism group itself is known to be the first of the five simple continuous groups which are not contained in the four general classes of simple continuous groups; our statements facilitate the study of the elementary properties in the direction of Lie's theory as well as in the direction of Thomsen's group geometry.[4]

The point of departure for these investigations was Brandt's theorem concerning automorphic transformations, the aim of the paper is to present a handy apparatus to any one who wishes to develop more complete theories in this domain.

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[1] Max Zorn, "Alternativkörper und quadratische Systeme Abh.," *Math. Sem. Hamburg*, **9**, S. 395–402.

[2] Encyclopédie des sci. math., Tome I, vol. I, p. 467.

[3] L. Dickson, *Transactions*, **2**, p. 383–391.

[4] G. Thomsen, *Math. Zeitschr.*, **34**, S. 668–720.

ON A TOPOLOGICAL CHARACTERIZATION OF COMPACT CONVEX POINT SETS

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S. Ulam has surmised the following property: Every closed $(n-1)$-surface S_{n-1} in an n-dimensional euclidean space E_n $(n > 2)$ whose (proper) intersection with every linear subspace E_{n-1} is a closed S_{n-2}, is convex. In a recent paper J. Schreier[1] has given a proof of this hypothesis for three-dimensional space; however, it is easily seen by an example that the hypothesis is not correct for $n > 3$. On the other hand, by a somewhat stronger condition which, to be precise, is actually not stronger in the case $n = 3$, we are able to state a general theorem characterizing the compact convex point sets by means of the topological behavior of their intersections with linear subspaces.

Let us start with some definitions. An open point set D in n-dimensional space is called *cell-like* if every p-cycle C in D,[2] $p = 0, 1, \ldots, n-1$, is the boundary of a chain in D. A point set P in E_n is said to be *point-like* if it is compact and its "spherical residual," $E_n^* - P$, is cell-like.[3] The theorem we are going to state is exactly the same whether the underlying coefficient domain for combinatorial chains is the system of all integers or the system of all integers mod m_0, m_0 a fixed integer > 1.

THEOREM. *A necessary and sufficient condition in order that a compact point set K in E_n be convex is the existence of an integer q, $0 < q < n$, such that the intersection E_qK of K with every linear subspace $E_q \subset E_n (E_qK \neq 0)$ is point-like.*

J. Schreier's result may be derived from this theorem.

The proof of it is based upon two lemmas of which only the first deals with combinatorial topology. Let us express briefly the condition of our

theorem by saying "*K is q-consistent in* E_n"; then these lemmas read as follows:

LEMMA 1. *If the compact point set K is q-consistent in* E_n, $0 < q < n$, *then for every supporting subspace* E_q *of K in* E_n[4] *the intersection* E_qK *is* $(q-1)$*-consistent in* E_q.

LEMMA 2. *Let K be a compact point set in* E_{m+1} *such that K does not separate* E_{m+1} *and for every supporting subspace* E_m *of K in* E_{m+1} *the intersection* E_mK *is convex. Then K is convex.*

Since 1-consistency is exactly the same as convexity, repeated applications of these lemmas give the theorem stated.

A simple argument is all that is needed to extend our theorem to Hilbert space.

[1] "Ueber Schnitte konvexer Flaechen," *Bull. int. Ac. pol.*, Series A, 155-157 (1933).

[2] I.e., the closure of C is contained in D.

[3] E^*_n is E_n made compact by addition of a point at infinity.

[4] An E_{n-1} $(n > 0)$ is an $(n-1)$*-dimensional supporting subspace of the compact point set K in* E_n if $E_{n-1}K \neq 0$ and one of the open half spaces in which E_n is divided by E_{n-1} contains no point of K. An E_q $(0 \leq q < n-1)$ is a *q-dimensional supporting subspace of the compact point set K in* E_n if there is a supporting E_{q+1} of K in E_n such that $E_{q+1} \supset E_q$ and E_q is a q-dimensional supporting subspace of $E_{q+1}K$ in E_{q+1}.

GENERALIZED CONCAVITY THEOREMS

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This paper is concerned with generalizations of the "Concavity Theorem," Morse ref. 1, p. 75. We shall refer to the general accessory boundary problem in the normal Bolza-Lagrange problem. The abnormal case can be similarly treated. Our theorems depend upon a Decomposition Theorem for quadratic forms which is a generalization of Lemma 7.4, p. 63, ref. 1. The mode of proof is an abstraction of the proof of the Concavity Theorem, p. 75.

1. *The Decomposition Theorem.*—Let $f(z)$ be a quadratic form in n variables (z). Let 0 be the point $(z) = (o)$. We shall consider hyperplanes π_r through 0 with subscripts r denoting the dimensionality. Suppose π_r has the form $z_i = a_{ij}u_j$ where $j = 1, \ldots, r$. On π_r, f *defines* a form $q(u)$. We shall denote the index and nullity of q by $i(q)$ and $v(q)$, respectively. Let $v(q, f)$ denote the dimension of the hyperplane of all critical points common to f and q.

The t-Plane Complementary to π_r.—A point (z) at which the directional

derivatives of f are null in all directions parallel to π_r will be termed a point complimentary to π_r. The set of all points (z) complimentary to π_r will form a t-plane π_t termed *complimentary* to π_r. The relation between π_r and π_t is invariant with respect to non-singular, homogeneous, linear transformations. Our basic theorem on quadratic forms is the following:

DECOMPOSITION THEOREM. *If $q(u)$ and $p(v)$ are respectively forms defined by f on π_r and the complimentary hyperplane π_t,*

$$i(f) = i(q) + i(p) + v(q) - v(q, f) \tag{1.1}$$
$$v(p) = v(q) + v(f) - v(q, f). \tag{1.2}$$

2. *The Functional I.*—We consider the functional I (η, u, o) of p. 523 of ref. 2, subject to end conditions (4.6) of p. 523. To apply the Decomposition Theorem we take $f(z)$ as the index form $Q(z, o)$ of (10.2), p. 531. The preceding r-plane π_r corresponds to a set of conditions on the vertices of broken extremals defining $Q(z, o)$.

We begin with a few definitions. A broken secondary extremal which satisfies the end conditions will be termed an admissible extremaloid. Let A be a class of admissible extremaloids. Suppose H is a finite set of extremaloids of A which contains the maximum number of linearly independent extremaloids every proper linear combination of which belongs to A. We term H a *maximal set* of extremaloids of A, and the number of extremaloids in H the *count* of extremaloids in A.

Let the columns of a matrix $\| \eta_{ij}(x) \|$, where $j = 1, \ldots, t$, represent the extremaloids of H. The set H defines a family

$$\eta_i = \eta_{ij}(x)v_j \quad (i = 1, \ldots, \eta;\ j = i, \ldots, t). \tag{2.1}$$

Upon evaluating the functional I along the extremaloids of the family (2.1) one obtains a form $q(v)$. We say that I *defines* $q(v)$ on the class A. In particular a form $p(v)$ defined by I on the class of admissible secondary extremals is termed a *Mayer end form*. The index of a Mayer end form is independent of the representation (2.1) used to define it, and will be termed the *order of concavity* of I.

Recall that a characteristic extremal is an admissible non-null extremal which satisfies the secondary transversality conditions. With this understood the Concavity Theorem of ref. 1 generalizes as follows.

THEOREM 2.1. *The index of I equals the number of conjugate points of a^1 on $a^1 < x \leqq a^2$, plus the order of concavity of I, minus the count of characteristic extremals which vanish at a^1 and a^2. The nullity of a Mayer end form equals the count of extremals which vanish at a^1 and a^2, plus the count of characteristic extremals which do not vanish at a^1 and a^2.*

This theorem, in the case where zero is not a characteristic root, was communicated to Dr. Hestenes in 1933. Recently Birkhoff and Hestenes have announced an independent proof of a theorem similar to the first

statement of the above theorem. See ref. 4 and the condition $m = r + s$ in abstract 173.

The case in which the end conditions involve two sets of distinct parameters and the end form $b_{hk}u_hu_k$ is a sum of two forms in these two sets of parameters, is called the case of *separate end parameters*. In this case let c be an arbitrary number between a^1 and a^2. Let

$$\begin{aligned} \eta_i &= \eta_{ij}(x)w_j \\ & \qquad (j = 1, \ldots, t) \qquad (2.2) \\ \zeta_i &= \zeta_{ij}(x)w_j \end{aligned}$$

be the family of extremaloids dependent on a maximal set of t admissible extremaloids which satisfy the transversality conditions and have intermediate vertices at most when $x = c$. On (2.2) I defines the concavity form

$$H(w) = [\eta_{ih}\zeta_{ik}]_{c^+}^{c^-}w_hw_k \qquad (h\ k = 1, \ldots, t). \qquad (2.3)$$

Our Decomposition Theorem leads to the following.

THEOREM 2.2. *In the case of separate end parameters the index of I equals the number of focal points, relative to I, of the end hyperplanes on $a^1 < x \leqq c$ and $c \leqq x < a^2$, respectively, plus the index of the concavity form* (2.3), *minus the count of characteristic extremals vanishing at $x = c$. The nullity of the form* (2.3) *equals the sum of the orders of c as a focal point of the two end hyperplanes, plus the count of characteristic extremals which do not vanish at $x = c$. See form H, ref.* 1, *pp.* 66–67.

We can apply the preceding theorem to a family F of mutually conjugate secondary extremals. With the preceding constants c and a^2 given, choose a^1 so near c that there are no focal points of F on $a^1 \leqq x < c$. Choose the end conditions so that $(\eta) = (o)$ at $x = a^2$, and at $x = a^1$ take the end conditions and end form so that the transversality conditions at $x = a^1$ define F. The form $H(w)$ given by (2.3) is well defined and we have the corollary.

COROLLARY. *The number of focal points of F on $c < x < a^2$ equals the number of conjugate points of a^2 on $c \leqq x < a^2$, plus the index of $H(w)$, minus the count of extremals of F vanishing at c and a^2.*

Theorem 2.1 can be generalized as follows. Let $b_1 < b_2 < \ldots < b_{p+1}$ be a set of numbers such that $b_1 = a^1$ and $b_{p+1} = a^2$. Let k_i be the number of conjugate points of b_i on $b_i < x \leqq b_{i+1}$, and h_i the index of b_{i+1} as a conjugate point of b_i. Let $p(v)$ be the form defined by I on the class of admissible extremaloids whose vertices lie only on the n-planes $x = b_i$.

GENERALIZED CONCAVITY THEOREM.—*The index of I equals $k_1 + \ldots + k_p$, plus the index of $p(v)$, minus the count of characteristic extremals which vanish at all the points $x = b_i$. The nullity of $p(v)$ equals $h_1 + \ldots + h_p$, plus the count of characteristic extremals which do not vanish at all of the points $x = b_i$.*

If $p = 1$ this implies Theorem 2.1. Theorem 2.2 can be similarly generalized.

[1] Morse, "The Calculus of Variations in the Large," Colloquium Lectures, *Amer. Math. Soc.*, New York.

[2] Morse, "Sufficient Conditions in the Problem of Lagrange with Variable End Conditions," *Amer. Jour. of Math.*, **53** (1931).

[3] Morse and Pitcher, "On Certain Invariants of Closed Extremals," these PROCEEDINGS, **20**, pp. 282–287 (1934).

[4] Birkhoff and Hestenes, "Abstracts 143 and 173," *Bull. Amer. Math. Soc.*, **41** (1935).

A THEOREM ON EXTREMALS. II

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5. In a previous Note under the same title[5] we gave a theorem on the foci of extremals. We continue our investigation and carry it to the point where we have a solid analytical foundation for Morse's type number theorems. The characteristic feature of our treatment is the complete avoidance of Jacobi's differential equations and secondary extremals.

We first consider another and better approach to the question discussed in No. 4. We may choose coördinates such that wherever considered the arcs γ^* are segments parallel to the x axis and such that x is the integral-length along γ^* from $x = 0$. Moreover the terminal manifold M_n^* may be taken as $x =$ constant. Under these circumstances, in the region about g that interests us, we find by means of the Euler equations and transversality that

$$f = 1 + D_{ik}(x)y_i'y_k' + \ldots, \tag{8}$$

where the quadratic form D is positive definite along g. We merely have to show that for $x_0 < 0$, $\bar{x}(t) = x(t) - x_0$ begins with a term αt^2, $\alpha < 0$, where t is as in (7) and x_0 is the abscissa of A. But $\bar{x}(t)$ is merely the x along the intersection of M_n with (7) as t varies. By transversality

$$\frac{d\bar{x}}{dt} = x_0 t(2D_{ik}(x_0)\mu_i\mu_k + \ldots) + \ldots, \tag{9}$$

where terms of higher order in $\bar{x}$, t combined are unwritten. The solution of this differential equation is

$$\bar{x} = x_0(2D_{ik}(x_0)\mu_i\mu_k)t^2 + \ldots \tag{10}$$

which is <0 for $x_0 < 0$. This proves our assertion. Moreover, the solution is valid for A anywhere on the open arc $B^{-1}A^1$.

Remark.—We nowhere exclude $k = 0$ (i.e., A^1 not a true focus).

7. The extremal pencils being now as in No. 1, we cut g in points $C^i (i = 1, 2, \ldots, r)$ by manifolds M_n^i transversal to $\{\gamma\}$, and we assume now merely that: (*a*) $C^iA^iC^{i+1}$ follow in the order named; (*b*) C^i has no conjugates on the closed arc $C^{i-1}C^{i+1}$; (*c*) $B^{-1}C^rA^rBB^1A^{r+1}$ follow in the order named. We take a point P^i very near C^i on M_n^i, and draw a γ^* through P^r meeting M_n^* in Q. Under the circumstances there are unique minimizing extremals P^iP^{i+1} and a unique arc P^rQ of γ^*, so that the integral-length of

$$F(P, Q) = \overline{P^1P^2} + \ldots + \overline{P^rQ} - \overline{C^1B} \tag{11}$$

is an analytic function of coördinates on the M_n^i about the points C^i.

THEOREM. $h = \text{index } F(P, Q) = \Sigma k_i$.

Outline of Proof.—We restrict the parameters (*a*) in (2) to an analytical cell $E_n \supset (a) = (0)$ (the parameter-point of g in $\{\gamma\}$). The intersections of the corresponding γ's with M_n^i make up a certain $E_n^i \supset C_i$, so that the (a)'s in question may serve as coördinates about C^i on M_n^i, and we impose $P^i \subset E_n^i$. Under the circumstances there is a unique γ^i, represented by a point $(a^i) \subset E_n$, containing P^i, and γ^{i+1} cuts M_n^i in $P_n'^i \subset E_n^i$. If $b_j^i = a_j^i - a_j^{i+1}$ $(a_j^{r+1} \equiv 0)$ the general extremal through P^iP^{i+1} near g is represented by an analytical system

$$y_i = y_i(x, a^{i+1}, b^i) \tag{12}$$

which reduces to the nature of (2) for $(b^i) = 0$. Hence

$$\overline{P^iP^{i+1}} - \overline{C^iC^{i+1}} = \phi_2^i(b^i) + \phi_3^i(a^{i+1}, b^i).$$

Taking $(a^{i+1}) = (0)$ with $\{\gamma^*\}$ replaced by the extremal pencil issued from A^{i+1}, or taken directly, we find by the theorem of No. 1 that ϕ_2^i is non-degenerate of index k_i. Hence $h = \text{index } \Sigma\phi_2^i = \Sigma k_i$.

8. *Concavity-Index.*—Let M_n, M_n^* be tangent in a point O regular for both. Choose coördinates x, y such that their equations about O become

$$x = \phi_2(y) + \ldots, x = \phi_2^*(y) + \ldots.$$

We call $h = \text{index } (\phi_2^* - \phi_2)$ the concavity-index of M_n^* relative to M_n in O. If $\phi_2^* - \phi_2$ is degenerate we say that the contact is *singular* in O. These notions can be extended to any pair M_p, M_q^* such that osculant $M_p \supset$ osculant M_q^*, but we use only $p = n$. In particular if $q = 0$ we agree that $h = 0$.

9. *Index of Extremals.*—Consider the following general situation: M_p, M_q^* are transversal to g in two points A, B. Let $\{\gamma\}$ be the transversal pencil to M_p. We carry out our construction as before except that $P^1 \subset M_p$. Let α, π, λ be the integral-lengths of AB, of the extremal polygon,

and of the arc intercepted by the terminals on the $\gamma \supset P'$. Let M_n^0 be the transversal to γ through B. If M_n^0 and M_q^* have non-singular contact in B, and concavity-index h we find

$$\text{index } (\pi - \lambda) = \Sigma k_i, \quad \text{index } (\lambda - \alpha) = h,$$

from which we then deduce:

THEOREM. *The index of non-degenerate non-closed extremals is* $h + \Sigma k_i$.

Clearly the rôle of the terminal manifolds may be interchanged. Furthermore the degenerate case may be treated in a similar manner.

COROLLARY: The Jacobi sufficiency conditions for two variable end-points are: (*a*) no focal points; (*b*) contact of M_n^0 and M_q^* to be non-degenerate; (*c*) $h = 0$.

Closed Extremals.—Let g be closed and with at least one point C not its own conjugate (of some order) as g is described once positively. Take for terminals $M_n = M_n^*$ an extremal-sphere of center C meeting g (forward) in A very near C. The polygon π is now closed with one vertex P on M_n, and P may be joined to itself around g by an extremal arc λ very near g varying continuously in a pencil $\{\delta\}$ as P varies on the sphere. Draw on λ, extended if need be, a positive arc $\overline{PQ}$ = integral-length of g. Its end-point Q describes an M_n^0 tangent to M_n in A, serving now to determine the concavity-index,[6] and the rest is as before.

[5] In the preceding number of these PROCEEDINGS. For convenience the numbering in the two notes is made consecutive throughout.

[6] Morse has defined analytically a concavity-index for closed extremals (loc. cit., p. 74). It is doubtless expressible as an index of our type but in what manner is not clear to us at the present time.

A CERTAIN REGION IN EUCLIDEAN 3-SPACE

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The object of this note is to describe informally a certain three-dimensional manifold for which a formal construction is to be given elsewhere.

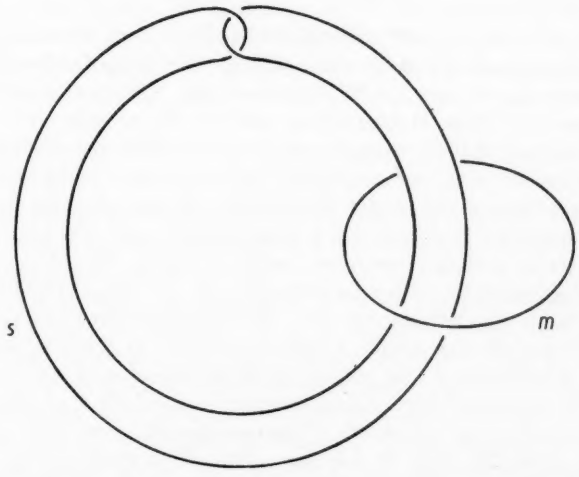
By a *ring* is meant a bounded three-dimensional manifold which can be cut into a 3-element along a suitable 2-element. By an unknotted ring R, in a 3-sphere H, is meant a ring such that $H-R$ is also a ring. A *simple self-linking* circuit in a ring R will mean an unknotted circuit s, of the type indicated by the diagram, R being the residual space (in the combinatorial sense) of an unknotted circuit m, in a 3-sphere H. It is obvious from the

diagram, and is the basis of the formal definition, that the circuit s bounds a singular 2-cell in R. An experiment with string leaves little room for doubt that:

1. There is no 3-element containing m and not any part of s;
2. The circuits m and s are interchangeable by a non-singular deformation.

From this second property it follows that any circuit in the ring $H-R$, $H-R$ being a tubular neighborhood of m, bounds a singular 2-cell in the residual space of s.

The manifold in question is equivalent to a complex covering the region $M = \Sigma - X$, where X is the set of points common to an infinite sequence of rings $T^0, T^1, \ldots$, defined inductively as follows. The first one, T^0, is any unknotted ring in a geometrical 3-sphere Σ, and T^{n+1} is a thin tube enclosing a simple self-linking circuit in T^n.



Any closed point-set in M is contained in $\Sigma - T^n$ for some value of n. Therefore any finite 2-cycle in M bounds a finite region in M. It follows from the properties of m and s that any circuit in $\Sigma - T^n$ bounds a 2-cell in $\Sigma - T^{n+1}$. Therefore the group of M is unity. If a Euclidean standard of linearity is assumed for Σ, and if $T^0, T^1, \ldots$ are polyhedral, it also follows that any non-singular polyhedral 2-sphere in M bounds a 3-element.

The question arises whether or not M is a 3-cell. It follows from the arguments given elsewhere that no rectilinear, simplicial covering of M is what I call a *formal 3-cell*. A formal 3-cell is defined as an infinite combinatorial complex which, after a suitable subdivision, is covered by an infinite sequence of 3-elements $E^1, E^2, \ldots, E^{n+1}$ containing every component

which meets E^n. It is not difficult to see that M is a formal 3-cell if, and only if, it is in a (1–1) semi-linear correspondence with Euclidean 3-space.

Were M a 3-cell, that is to say, homeomorphic to Euclidean 3-space, a rectilinear covering of the former would determine a remarkable simplicial covering of the latter. It would be seen that the "Hauptvermutung" was at least false for infinite complexes.

ON NORMAL OPERATORS

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1. In a previous paper* the notion of normalcy was extended to non-bounded operators of Hilbert space ((2), p. 406; as to further literature about normalcy cf. eod., p. 377, footnote 25). The notions which are fundamental in those investigations, and for the present ones too, are these: Abstract Hilbert space $\mathfrak{H}$; the set of all linear bounded operators in $\mathfrak{H}$, $\mathbf{B}$; the set of all (not necessarily bounded) linear closed operators in $\mathfrak{H}$, $\mathbf{C}$; the notion of the adjoint R^* for $R \epsilon \mathbf{C}$; the notion of commutativity for two operators, R, A, where R is arbitrary and $A \epsilon \mathbf{B}$; the set M' where M is a set of arbitrary operators, and the iterates M'', M''', . . .; the notion of a ring in $\mathbf{B}$. They are defined in (2), pp. 388–389 and 404–405, and in (4), pp. 294–295 and 300–301. We emphasize the following facts:

$\mathbf{B} \subset C$; if $R \epsilon \mathbf{B}$ then $R^* \epsilon \mathbf{B}$; if $R \epsilon \mathbf{C}$ then $R^* \epsilon \mathbf{C}$. If R is arbitrary, $A \epsilon \mathbf{B}$, then R, A commute if and only if RA is an extension of AR. In other words: $f \epsilon$ Domain R implies $Af \epsilon$ Domain R and $R(Af) = A(Rf)$. M' is the set of all A, for which A, A^* commute with every $R \epsilon M$. Always $M' \subset \mathbf{B}$; if $M \subset \mathbf{C}$, then M' is a ring. Thus M'' is always a ring. A ring M (which must by definition be a subset of $\mathbf{B}$) is Abelian if all its elements commute, that is $M \subset M'$.

It would be feasible to define normalcy for the operators $R \epsilon \mathbf{C}$ only, but it means no additional work to extend this domain somewhat. We will consider such operators R for which R^* exists and has an everywhere dense domain. Then $R^* \epsilon \mathbf{C}$. An equivalent condition is that there exists an extension S of R, $S \epsilon \mathbf{C}$; this extension can then be chosen as the closure of R, $S = \tilde{R}$ ((4), p. 301). For $R \epsilon \mathbf{C}$ we have, of course, $\tilde{R} = R \epsilon \mathbf{C}$, $R^* \epsilon \mathbf{C}$. (In other words: $R^* \epsilon \mathbf{C}$ is a weaker condition than $R \epsilon \mathbf{C}$, and suffices for our purposes.) By an obvious transformation, the definition of normalcy which was given, loc. cit., can be formulated as the sum of the two following conditions:

(α) The ring $(R)''$ is Abelian.

(β) The intersection Domain $R \cdot$ Domain R^* is everywhere dense.

Considering the pathological possibilities connected with the domain of non-bounded operators ((3), pp. 230–234, and (1), pp. 422–427) it seemed advisable to insist on (β) or some similar condition. The results of (4) however show that unexpected relations exist between Domain R and Domain R^*, and thereby suggest that (β) may be unnecessary.

We propose to show in this note that this is the case: We will see that (α) implies Domain $R \subset \mathbf{C}$ Domain R^*, and so (β) and normalcy. (Alternative definitions of normalcy were given in (4), p. 309.)

2. Following the procedure indicated above, we define:

Definition: R is normal (in the new sense), abbreviated: n. n. s., if $R^* \epsilon \mathbf{C}$ and $(R)''$ is Abelian.

If A commutes with R, then A^* commutes with R^* ((2), pp. 406–407), assuming $A \epsilon \mathbf{B}$; so if A, A^* commute with R, they commute with R^* too ($A \epsilon \mathbf{B}$ implies $A^{**} = A$). Thus $(R^*)' \supset (R)'$, $(R^*)'' \subset (R)''$, and so $(R^*)''$ is Abelian along with $(R)''$. Besides $R^{**} = \tilde{R} \epsilon \mathbf{C}$, so R^* is n. n. s.

If $R \epsilon \mathbf{C}$ and R^* n. n. s., then $R = \tilde{R} = R^{**}$ is n. n. s., too. So we have proved:

THEOREM 1. *If R is n. n. s., then R^* and $\tilde{R}$ are n. n. s., too. If $R \epsilon \mathbf{C}$ then R is n. n. s. if and only if R^* is n. n. s.*

This theorem shows that in considering n. n. s. operators we can restrict ourselves to those in **C**: Every other n. n. s. R can be replaced by $\tilde{R}$.

3. Assume now $R \epsilon \mathbf{C}$, R n. n. s.

Apply the fundamental Theorem 7 in (4), p. 307 to R (denoted there by A), and form the operators B, W as described there. If U_0 is any unitary operator, that is any isomorphism of Hilbert space $\mathfrak{H}$, then the connection between R, B, W is invariant under the transformation U_0. In other words, as R determines B, W uniquely: If we replace R by $U_0^{-1} R U_0$, then B, W become $U_0^{-1} B U_0$, $U_0^{-1} W U_0$. So if R is invariant under the transformation U_0, then B, W are too. Now let U be the Cayley transform of B ((1), pp. 80–82), as B is hypermaximal, U is unitary ((1), p. 88). As B is invariant under U_0, U is it too. So every unitary $U_0 \epsilon (R)'$ belongs to $(W, U)'$, in the notation of (2), p. 392: $(R)'^{\mathfrak{u}} \subset (W, U)'^{\mathfrak{u}}$. Both $(R)'$ and $(W, U)'$ are rings containing 1, so by the theorem loc. cit. they are the rings generated by $(R)'^{\mathfrak{u}}$, resp. $(W, U)'^{\mathfrak{u}}$. Thus $(R)' \subset (W, U)'$, $(R)'' \supset (W, U)''$. Therefore $(W, U)''$ is Abelian, along with $(R)''$.

As $(W, U) \subset \mathbf{B}$, therefore $(W, U) \subset (W, U)''$ ((1), p. 388). So $W, U \epsilon (W, U)''$, and as $(W, U)''$ is a ring, $W^* \epsilon (W, U)''$. Therefore W^*, U commute.

By (1), p. 81, Domain B consists of all $f = U\varphi - \varphi$. Then $W^* f = W^* U\varphi - W^*\varphi = UW^*\varphi - W^*\varphi = U\psi - \psi$ with $\psi = W^*\varphi$, so $W^* f \epsilon$

Domain B. In other words: Domain (WB) = Domain $B \subset$ Domain (BW^*). Now by the theorem in (3), p. 307, quoted above, $R = WB$, $R^* = BW^*$. So Domain $R \subset$ Domain R^*.

By Theorem 1, R^* too is n. n. s. and $R^* \epsilon \mathbf{C}$, so Domain $R^* \subset$ Domain R (remember $R^{**} = \tilde{R} = R$). Thus Domain R = Domain R^*.

If we assume R n. n. s. only, then by Theorem 1, R^* n. n. s., $R^* \epsilon \mathbf{C}$. So we have Domain $R \subset$ Domain $\tilde{R}$ = Domain R^{**} = Domain R^*. This proves:

THEOREM 2. *If R is n. n. s., then Domain $R \subset$ Domain R^*; if $R \epsilon \mathbf{C}$, R n. n. s., then even Domain R = Domain R^*.*

We now see that the intersection Domain $R \cdot$ Domain R^* is equal to Domain R, and therefore everywhere dense. The pairs R, R^*, as discussed in (2), pp. 405–406, can therefore be derived from our n. n. s. operators R. It is to be noted, however, that the R^* occurring loc. cit., which we will denote here by R_1^*, is not our present R^*. R_1^* arises by restricting the domain of R^* to Domain R (which is $\subset$ Domain R^*). Thus R^* is an extension of R_1^*. As $\tilde{R}$ is an extension of R, and Domain $\tilde{R}$ = Domain R^{**} = Domain R^*, Domain R_1^* = Domain R, therefore, $\tilde{R} = R$ and $R^* = R_1^*$ are equivalent. In other words: R_1^* coincides with R^* if and only if $R \epsilon \mathbf{C}$ (assuming R n. n. s.).

Thus the considerations of (2), pp. 406–417, apply to all n n. s. operators and our notion of normalcy (n. n. s.) is equivalent to the one used loc. cit. Our Theorem 2 permits even a simplification of those considerations, because it gives immediately the fact (which is of importance there) that every $R \epsilon \mathbf{C}$ which is n. n. s. and Hermitian, must be hypermaximal (self adjoint): As R is Hermitian, R^* is an extension of R; as $R \epsilon \mathbf{C}$ and R n. n. s., therefore, Domain R = Domain R^*; these two statements combined give $R = R^*$, that is the hypermaximality of R.

4. Let R, S be n. n. s., R an extension of S. Then S^* is clearly an extension of R^* (the conditions defining S^*f are part of those defining R^*f), and $R^{**} = \tilde{R}$ is an extension of $S^{**} = \tilde{S}$. Thus Domain $R^* \subset$ Domain S^*, Domain $R^{**} \supset$ Domain S^{**}. But Theorem 2 gives, for R^* and S^*, that Domain R^* = Domain R^{**}, Domain S^* = Domain S^{**}. Thus Domain R^{**} = Domain S^{**}, and therefore $R^{**} = S^{**}$, $\tilde{R} = \tilde{S}$.

If R, $S \epsilon \mathbf{C}$, we can even write $R = S$.

So we have proved:

THEOREM 3. *If R, S are n. n. s., R an extension of S, then $\tilde{R} = \tilde{S}$. Therefore, no n. n. s. element of $\mathbf{C}$ can be a proper extension of another one.*

This theorem shows that $\tilde{R}$ is the maximal n. n. s. extension of a given n. n. s. operator R. Therefore it is the $\overline{R}$ which was defined in (2), pp. 409–410, in a different way.

* In this note definitions and results from four previous papers of the author will be used. They will be referred to by current number, as follows:

(1) "Allgemeine Eigenwerttheorie Hermitescher Funktionaloperatoren," *Math. Ann.*, **902**, 49–131 (1929).

(2) "Zur Algebra der Funktionaloperatoren und Theorie der normalen Operatoren," *Ibid.*, **102**, 370–427 (1929).

(3) "Zur Theorie der unbeschränkten Matricen," *Jour. Math.*, **161**, 208–236 (1929).

(4) "Uber adjungierte Funktionaloperatoren," *Ann. Math.*, **33**, 294–310 (1932).

CHROMOSOME ENDS IN DATURA DISCOLOR

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In 1932[1] we reported on the chromosomes involved in the configurations found in the *Datura stramonium-quercifolia-ferox* triangle. Because cryptic chromosomal types (prime types) have been found by means of *intra se* crosses, the precaution was taken to use one race only as a standard for each species. These tester races when examined cytologically showed their 24 chromosomes arranged as 12 bivalents at metaphase I in pollen-mother-cells. The results obtained therefore were limited to these tester races; different results would have been obtained by using certain other races as testers. In the present paper we are adding *D. discolor* to the list of those species of *Datura*, the chromosomes of which have been given numerical representation in terms of those of our standard Line 1, the tester race in *D. stramonium*. In these analyses we are interpreting the end arrangements of other species in terms of those of this Line 1 standard. In describing chromosomes in *D. color* as modified with respect to those of Line 1 as a result of segmental interchange, we do not wish to imply that Line 1 is the primitive type from which other races and species have developed.

D. discolor is a species of the southwestern states and Mexico. It has nodding capsules and arils on the seeds, characters which are not present in the *Stramonium* group. Its taxonomic affinities, therefore, would appear to be with the group including *D. meteloides*, *D. innoxia* and *D. leichhardtii*. So far as crossability is concerned, however, *D. discolor* shows clearly relationship with species in the *Stramonium* section. Hybrids have been obtained with *D. stramonium*, *D. ferox* and *D. quercifolia*, provided *D. discolor* is used as the male parent.

The 24 chromosomes of the *D. stramonium* x *D. discolor* hybrid at metaphase I in *PMC* were arranged as a circle of ten chromosomes plus

seven bivalents. Those of the *D. ferox* x *D. discolor* hybrid were arranged as a circle of ten chromosomes (or a circle of six to which a circle of four was attached) plus seven bivalents, including one unequal pair. Those of the *D. quercifolia* x *D. discolor* hybrid were arranged as a circle of six chromosomes plus a circle of four plus seven bivalents, including one unequal pair. Drawings of these arrangements are shown in figure 1. Since all circles show a tendency to be broken at particular fixed points, these configurations are more frequently found as chains than as circles.

FIGURE 1

Sizes are indicated for all bivalents except the unequal pairs. In *b* a dotted line connects two univalents making an *l*-sized bivalent which usually was closed in other *PMC*.

As far as their ends are concerned, seven of the chromosomes in *D. discolor* are the same as seven in Line 1 in *D. stramonium*, but the other five are different. In order to identify these five, a study was made of the chromosome arrangements in crosses to selected prime type tester races[2] of *D. stramonium*. Since all the chromosomes of the tester races of *D. ferox* and *D. quercifolia* had been identified previously,[1] their arrangements in the *D. ferox-discolor* and in the *D. quercifolia-discolor* hybrids were studied in order to verify the determinations made by means of the crosses to prime type testers of *D. stramonium*.

A prime type is a race in which some of the chromosomes have altered end arrangements in comparison with those of a standard race. Their use in identifying chromosome ends has been described in a previous publication.[2] Crosses with prime types 4, 7, 11, 33, 34, 40 and 51 were studied in order to identify these five modified chromosomes of *D. discolor*. Table 1 gives a summary of the results.

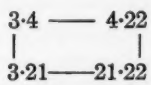
TABLE 1

Datura stramonium TESTER RACES USED FOR IDENTIFYING THE ENDS OF THE CHROMOSOMES OF *D. Discolor*

(a) PRIME TYPE	(b) MODIFIED CHROMOSOMES	(c) LINE 1 CHROMOSOMES INVOLVED	(d) CROSS WITH *D. discolor* SHOWS	(e) INTERPRETATION OF CONFIG.
4	3·21	3·4	Config. 12	One *discolor* chr. with ends similar to a L 1 chr. in (*c*)
	4·22	21·22		
7	$9{\cdot}10^{20}$*	$9{\cdot}10^{10}$	Config. 10 +	Two *discolor* chrs. with ends similar to L 1 chrs. in (*c*)
	$19{\cdot}20^{10}$	$19{\cdot}20^{20}$	Config. 4	
11	11·13	11·12	Config. 12	One *discolor* chr. with ends similar to a L 1 chr. in (*c*)
	12·17	13·14		
	14·18	17·18		
33	7·8·15	$7{\cdot}8^{8}$	Config. 12	One *discolor* chr. with ends similar to a L 1 chr. in (*c*)
	$16{\cdot}^{8}$	15·16		
34	$11{\cdot}12^{24}$	$11{\cdot}12^{12}$	Config. 12	One *discolor* chr. with ends similar to a L 1 chr. in (*c*)
	$23{\cdot}24^{12}$	$23{\cdot}24^{24}$		
40	5·20	5·6	Config. 10 +	Two *discolor* chrs. with ends similar to L 1 chrs. in (*c*)
	6·19	19·20	Config. 4	
51	$2{\cdot}^{12}$	1·2	Config. 10	No *discolor* chr. with ends similar to L 1 chr. in (*c*)
	11·12·1	$11{\cdot}12^{12}$		

* The raised numbers represent humps on chromosomes. Humps are represented only where they are interchanged.

The cytological examination of two crosses will be given to explain the method used. Prime type 4 has two chromosomes, 3·21 and 4·22, which differ in their end arrangements from the 3·4 and 21·22 chromosomes in Line 1. The F_1 between them shows, in addition to ten bivalents, a circle of four chromosomes which may be represented as follows:



The hybrid, Line 1 *stramonium* x *D. discolor*, showed a circle of 10 chromosomes. The hybrid, *PT* 4 *stramonium* x *D. discolor*, however, showed a circle of 12 chromosomes; that is, when the two modified chromosomes from *PT* 4, 3·21 and 4·22, were substituted for the Line 1 chromosomes, 3·4 and 21·22, the size of the circle was increased by two chromosomes and

the number of bivalents was decreased by one. Since the number of bivalents was decreased from seven to six, one more chromosome from *D. discolor* was brought into the circle of twelve (*PT* 4 x *discolor*) which in the hybrid Line 1 x *discolor* was in a bivalent. Since this chromosome connected the two modified chromosomes of *PT* 4, 3·21 and 4·22, it was either the 3·4 or the 21·22 chromosome. The 3·4 chromosome is l-sized, the 21·22 is S-sized, the 3·21 is S-sized and the 4·22 is l-sized. Since in the circle of twelve there were present two l-sized chromosomes not present in the circle of ten, these were the 3·4 and 4·22 chromosomes. Therefore the 3·4 chromosome formed a bivalent in the Line 1 x *discolor* hybrid and the ends ·21 and ·22 were represented on two different chromosomes among the five modified chromosomes of *D. discolor*.

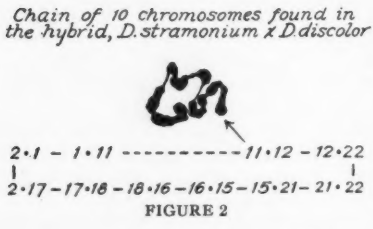


FIGURE 2

Having the same chromosome represented in the interchange involved in several prime types helps in making identifications. For instance, the ends of the 11·12 chromosome were tested in *PT*'s 11, 34 and 51.

By means of *PT* 7, the ends of the 9·10 and 19·20 chromosomes of Line 1 were tested. Since the *PT* 7 *stramonium* x *discolor* hybrid showed a circle of ten chromosomes plus a necktie of four (*PT* 7 in the heterozygous condition) both the 9·10 and the 19·20 chromosomes were in bivalents in the Line 1 x *discolor* hybrid.

By means of the different crosses, it was learned that the five chromosomes of Line 1 which entered into the formation of the circle of ten in the Line 1 *stramonium* x *discolor* hybrid were the 1·2, 11·12, 15·16, 17·18 and 21·22 chromosomes.

The numerical representation of the five modified *discolor* chromosomes was possible only after both ends of each had been identified. Morphological peculiarities of the chromosomes of the tester races, such as size, shape or the position of a terminal hump, enable one to locate them in a sequence of several attached chromosomes (circle, chain or necktie). Based on the assumption that like ends of chromosomes are attached, it is then a simple task to write out the formula of a chromosomal configuration. Since morphological peculiarities also distinguish the modified chromosomes of *discolor*, their identification was completed. These five *discolor* chromosomes are 1·11, 2·17, 12·22, 15·21 and 16·18. Figure 2 shows a drawing of the chain (broken circle) of ten chromosomes found in the *D. stramonium-discolor* hybrid, with their numerical representation underneath. The dotted line indicates the place where the circle was broken.

These modified chromosomes of *D. discolor* which differ from those in Line 1 of *D. stramonium* are listed in table 2 along with those of *D. ferox* and *D. quercifolia* which were published previously.[1] The order of the *stramonium* chromosomes has been arranged to emphasize the fact that the same modified chromosomes may be found in different species. For

TABLE 2

CHROMOSOMES INVOLVED IN THE CONFIGURATIONS FOUND IN STRAMONIUM-FEROX-QUERCIFOLIA-DISCOLOR HYBRIDS

DATURA STRAMONIUM	D. FEROX	D. QUERCIFOLIA	D. DISCOLOR
1·2	1·18	1·18	1·11
17·18	2·17	2·17	2·17
21·22	12·22	12·22	12·22
11·12	11·21	11·21	15·21
15·16	$15{\cdot}16^{20}$*	15·16	16·18
7·8	$7{\cdot}20^{16}$	7·20	7 8
19·20	8·19	8·19	19·20

* The raised numbers represent humps on chromosomes. Humps are represented only where they are interchanged. The order of the stramonium chromosomes has been arranged to show that, as far as the ends of chromosomes are concerned, the same modified chromosomes may be found in different species.

example, the 2·17 and 12·22 chromosomes occur in *D. ferox*, *D. quercifolia* and *D. discolor*. The 2·17 chromosome has been found also in the tester race of *D. leichhardtii* as well as in many races of *D. stramonium*. However, these chromosomes may not be identical throughout their entire length since only their ends have been determined. The 12·22 chromosome in *D. discolor* is larger than the 12·22 chromosome in either *D. ferox* or *D. quercifolia*. This difference is shown in the hybrids, *D. ferox* x *discolor* and *D. quercifolia* x *D. discolor*, where the 12·22 bivalents consist of unequal-sized chromosomes (figure 1, *a* and *b*). Apparently different interchanges are responsible for the production of the 12·22 chromosome in the different species.

In all interspecific hybrids involving eight species of the genus *Datura* so far examined, the chromosomes at metaphase I were arranged either as bivalents or were attached in configurations which involved four or more chromosomes. While it is true that attachments between certain like ends of chromosomes from different species may be weak, no real failure of chromosomes to pair or to become attached in configurations has been found. No new attachment ends have been observed. The same twenty-four ends found on the twelve chromosomes of the standard Line 1 of *D. stramonium* have been identified by means of interspecific hybrids in these other species. In the derivation of these species from primitive types, some of these attachment ends have become located on

different chromosomes. The hypothesis of segmental interchange between non-homologous chromosomes will account for the formation of such modified chromosomes.

[1] Bergner, A. D., and Blakeslee, A. F., these PROCEEDINGS, **18**, 151–159 (1932).
[2] Bergner, A. D., Satina, S., and Blakeslee, A. F., *Ibid.*, **19**, 103–115 (1933).

THE EFFECT OF YELLOW-SCUTE GENE DEFICIENCY ON SOMATIC CELLS OF DROSOPHILA

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It is known that zygotes of *Drosophila melanogaster* cannot give rise to individuals unless each chromosome region is represented once at least. To this Demerec[1] has added the significant fact that even a somatic cell which, during development, acquired a constitution homozygous for a small deficient region is not able by division to give rise to a group of viable cells. Out of 11 regions of the *X*-chromosome, which were studied, all but one were found to be "cell-lethal" when absent. The one exceptional case is stated to produce only small and weakly developed cell areas. In contrast to these findings, Ephrussi,[2] who has studied two deficiencies, both including the left, yellow end of the *X*-chromosome, obtained mosaic hypodermal areas deficient for these chromosome regions and of apparently normal viability. Although the different findings of both Demerec and Ephrussi are compatible with the expectation that different chromosome regions may have different values for determining life processes it seemed desirable to repeat Ephrussi's experiments with a somewhat changed method.

Ephrussi studied males whose *X*-chromosome was deficient for the *y*-region but whose viability as individuals was assured by the possession of a fragment attached to the fourth chromosome and containing the genes absent in the deficient *X*-chromosome. This fragment frequently was "eliminated" (somatic segregation?) during development so that cells resulted which were deficient for the critical region of the *X*-chromosome. According to Ephrussi, these cells divided and gave rise to yellow patches on the normal black background of the fifth to seventh abdominal segment. The yellow patches were covered with setae of normal size and number although it is stated that only some of these setae were yellow while others were black. No comparison was possible between the viability of

the mosaic region and that of a comparable not deficient region. The method employed by the present author allows for an evaluation of the viability of deficient cells. The fact is used that in females somatic segregation of the *X*-chromosomes leads to two daughter cells with new genetic constitutions: one daughter cell will obtain the two sister-chromatids of one original *X*-chromosome, the other will obtain the two sister-chromatids of the other original *X*-chromosome.[3,4] Accordingly, a cross was made between $y\ Hw/(y\ sc)^-$ females and $sn^3M/+$ males (table 1). $(y\ sc)^-$ is the deficiency tested, which comprises the genes yellow (I,00) achaete (I,0.0+) and scute (I,0.0+) and is identical with one of the two which Ephrussi used ("deficiency 100, obtained by Mrs. L. V. Morgan").[5] *Hw* (Hairy wing, I,0.0+) is a dominant sex-linked gene; *M* is a dominant third chromosome Minute which is known to increase the frequency of somatic segregation. The F_1-females from the cross were either $y\ Hw/sn^3$ or $(y\ sc)^-/sn^3$ (disregarding *M*). The latter were completely wild type, the former showed the *Hw* phenotype. As a control about 266 $y\ Hw/sn^3$ females were inspected for mosaic spots. The color and shape of the setae anywhere on the surface of the flies served as a criterion. 121 spots were found, 43 of these were, according to expectation, twin spots made up of neighboring yellow and singed areas. Of the remaining 78 single spots about half were either only *y* or only sn^3. An inspection of the sizes of spots as estimated from the number of setae affected shows that these single spots were so small as to comprise only one seta in the majority of cases which obviously makes it impossible for them to be recognized as twin spots. In order to compare the viability of the *y* with that of the

TABLE 1

$y\ Hw/(y\ sc)^-\ ♀ \times sn^3M/+\ ♂$

CONSTITUTION OF F_1 FEMALES	INDIVIDUALS INSPECTED	NO. OF SPOTS	PHENOTYPE OF SPOTS & NUMBER OF SETAE: y 1	y 2	y >2	sn^3 1	sn^3 2	sn^3 >2	TWIN y sn^3 2	TWIN y sn^3 >2	TOTAL NUMBER OF SETAE IN TWIN	TOTAL NUMBER OF SETAE IN SINGLE sn^3	TOTAL NUMBER OF SETAE IN SINGLE y	GRAND TOTAL y sn^3
$\frac{y\ Hw}{sn^3}$	266+	121	33	6	3	24	7	5	9	34	89	107	49	164
			42			36			43					
$\frac{(y\ sc)^-}{sn^3}$	289+	130	5 + 1?	..	..	62	24	37	1?	..	1	1	6	7
			6			123			1			320	320	

sn^3 segregation-product the total number of y and sn^3 setae in the 43 twin spots was calculated. The proportion is 89 y: 107 sn^3 setae, the deviation from equality being 1.3 times the standard error. Another measure is given by a comparison of the sum of all y- and all sn^3-setae in not twin spots. Omitting two exceptionally large y and one such exceptional sn^3 spot there were 49 y: 57 sn^3 setae. Totaling the setae of all spots (with the exception of the three mentioned) we find 138 y:164 sn^3 (45.7%:54.3%). The difference from equality is only 1.6 times the standard error. There was then no significantly better viability (as measured by growth) of one or the other kind of cells produced by segregation.

A very different result was obtained from the $(y\ sc)^-/sn^3$ sisters of the controls. 130 mosaic spots were found on about 289 females. Only one single twin-spot was among these spots and there was some doubt if the one light colored seta next to the one singed seta was really y or only lightly colored on account of some accidental happening. There were only 6 y spots (one doubtful), each covering not more than one single seta. The rest of the 123 spots exhibited the sn^3-phenotype. In contrast to the sn^3-spots in the controls where only 12 out of 36 covered more than one seta, in the $(y\ sc)^-/sn^3$ flies, 61 out of 123 spots covered more than one seta. The total numbers of y and sn^3 setae in the $(y\ sc)^-/sn^3$ flies were, including the two doubtful y setae, 7 y: 320 sn^3. These results lead to the conclusion that the $(y\ sc)^-/(y\ sc)^-$ segregation product has an extremely low viability. No case has been found in which it grew sufficiently to cover more than one seta compared to 61 cases in which the sn^3 segregation product attained this size. In only 7 cases was the y-segregation product able to form the one single seta as compared with 123 cases in which the 320 sn^3-seta were formed.

The results agree with those of Ephrussi in indicating that cells deficient for the *y sc* region of the *X*-chromosome can survive and differentiate and that such cells produce *yellow* setae. They do not agree as to the grade of viability of deficient cells: Ephrussi reports the frequent formation of comparatively large deficient areas while in the present material such deficient areas are not only rare, but are also extremely small: probably confined to a single cell.

It seemed possible that the different results were correlated with the fact that Ephrussi found spots only on the fifth to seventh abdominal segment, while in the present experiment spots occurred all over the surface of flies. Table 2, however, shows that there were 40 sn^3 spots in the critical abdominal region, of which probably at least one-half occurred early enough to have given rise to yellow and singed twin spots if the environment represented by this region had been favorable to the survival of the $(y\ sc)$ deficient cells.

It might be argued that in the experiment described above only the

yellow color of setae was taken as identification for a mosaic spot while Ephrussi used the yellow color of the chitinous body wall. However, the yellow color of the hypodermis in small spots is generally so slightly different from the color of the surroundings except in the black regions of the male abdomen, that it could not be used in the present experiments which deals only with females. As Ephrussi had found both yellow and black setae on his patches it might be objected that the size of spots in the present experiment was underrated for the size estimate was only based on the yellow setae. Consequently a second experiment was performed, mating $(y\ sc)^{-}sn^{3}/y\ Hw\ dl$-49 females to Minute males which were neither

TABLE 2

DISTRIBUTION OF SPOTS (FROM TABLE 1) OVER DIFFERENT REGIONS OF THE BODY SURFACE

PHENOTYPE OF SPOT	HEAD	THORAX	ABDOMINAL SEGMENT 1 + 2	3	4	5	6	7
y	..	2	..	3	..	1	..	..
sn^{3}	2	23	4	24	28	25	9	6
y / sn^{3} twin	..	..	..	..	1	..	..	..

TABLE 3

$y\ Hw\ dl$-49$/(y\ sc)^{-}\ sn^{3}$ ♀ × $+\ M/+$ ♂

CONSTITUTION OF F_1 FEMALES	TYPE OF SPOT	INDIVIDUALS INSPECTED	NO. OF SPOTS	NO. OF SETAE IN SPOTS 1	2	>2	TOTAL SETAE
$\frac{y\ Hw\ dl\text{-}49}{+}$	y	600	136	91	15	30	
					136		252
$\frac{(y\ sc)^{-}\ sn^{3}}{+}$	$y\ sn^{3}$	603	17	17	...	..	
					17		17

y nor sn^{3} (table 3). On account of the presence of the dl-49 inversion there was no crossing-over in the left end of the X-chromosomes of the P-females thus leading to two types of F_1-females only: $y\ Hw\ dl$-$49/+$ and $(y\ sc)^{-}sn^{3}/++$. 600 females of the first and 603 of the second kind were inspected for mosaic patches. The control females ($y\ Hw\ dl$-$49/+$) were expected to give somatic segregation products of the constitution $y\ Hw\ dl$-$49/y\ Hw\ dl$-49 and $+/+$ leading only to y patches. 136 such spots were found covering one, two or more setae. The $(y\ sc)^{-}\ sn^{3}/++$ females were expected to form segregation products of the constitutions $(y\ sc)^{-}sn^{3}/(y\ sc)^{-}sn^{3}$ and $+/+$. All chaetogenous cells homozygous for sn^{3} are known to produce sn^{3} setae. If all these cells which in this case were also homo-

zygous for $(y\ sc)^-$ would show the yellow phenotype, only yellow singed setae were expected, and if the viability of (*y sc*) deficient cells was again as low as in the first experiment, only few yellow singed spots and these as single setae should occur. If, however, only some of the (*y sc*) deficient setae exhibited the yellow phenotype, then one would find spots containing yellow singed and singed setae. Only 17 spots were found each exhibiting one single (yellow singed) seta, making a total of 17 setae as compared with 252 setae in the 136 control spots.[6] This result again demonstrates the extremely low viability of (*y sc*) deficient cells in our experiments. It should be added that the size of setae produced by (*y sc*) deficient cells has been found to be extremely small in a number of cases, but in others a normal growth had occurred.[7]

The result of this investigation that cells deficient for certain genes are of very low viability holds an intermediate position between the extremes found by Demerec and Ephrussi. Different results for different deficient cells might be expected. It remains to be explained, however, why Ephrussi's and the results reported here show such differences, although obtained with the *same* deficiency. One difference between the two sets of experiments consists in that Ephrussi investigated males and 1 *X*-chromosome cells deficient for (*y sc*) while we investigated females and 2 *X*-chromosome cells deficient for (*y sc*). It seems not very likely, though possible, that this may account for the divergent results. A final explanation has perhaps to await a more detailed account of the experiment with males.

Ephrussi reports his results under the title "the absence of autonomy in the development of the effects of certain deficiencies..." and he stresses the same point in his conclusion "that groups of deficiency-carrying cells surrounded by cells of normal constitution are viable in the case of the two *X*-chromosome deficiencies studied." This deduction, however, is not the only one possible. It could very well be that the deficient regions are not necessary for the life and differentiation of hypodermal cells and that the deficient cells would have survived even without being surrounded by normal cells. A decision between the hypothesis of non-autonomous development or autonomy in spite of gene deficiency cannot be derived from the present data.

Summary.—Female cells homozygous for the (*y sc*) deficiency are rarely viable. No case was found in which the cells or their derivatives covered an area of more than one seta. These results are difficult to understand in the presence of results obtained by Ephrussi with the same deficiency.

[1] *Proc. Nat. Acad. Sci.*, 20, 354–359 (1934).

[2] *Ibid.*, 20, 420–422 (1934).

[3] The fact that the chromosomes have undergone crossing-over near the spindle fibre point can be left out of consideration in this connection.

[4] Somatic segregation thus generally does not lead to a reduction of the number of *X*-chromosomes—not to a "hemizygous" condition.

[5] Dr. Demerec kindly informs me that studies of salivary gland chromosomes show that this deficiency is cytologically visible and includes a region from the end of the chromosome up to and including 1B3 or 1B4 bands of Bridges' chart.

[6] These control spots of course occurred in sister flies of different constitution while in the first experiment there was also a comparison to be derived between the two complementary kinds of spots in individuals of the same constitution.

[7] Apart from the spots discussed above a few spots of different phenotypes were found. Most of them can be explained by somatic crossing-over between the *y* and *sn*^{3} locus and subsequent segregation. Besides these there occurred three patches of considerable size which were devoid of all setae. Their nature is under investigation.

HEADDOT: AN INCOMPLETELY RECESSIVE WHITE SPOTTING CHARACTER OF THE HOUSE MOUSE

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Three distinct types of inherited spotting in the house mouse are now recorded in the literature. The first to be investigated was recessive or belted piebald (Fig. 1, type 1) reported upon by Cuénot in 1902. Dominant spotting (anemic lethal) was reported by Durham in 1908 (Fig. 1, type 4). In 1934 Clark published upon a recessive belly spot type which is inherited together in a complex with flexures of the tail and a temporary infantile anemia (Fig. 1, type 3). Dunn and Durham in 1925 isolated from recessive piebald stocks a type called "white face" which they interpreted as due to the presence of belted piebald plus a modifier. A similar type known as Berlin blaze (Fig. 1, type 2) is now being studied at the Bussey Institution, but our investigation suggests that Berlin blaze is due to an allelomorph of the piebald gene rather than to the piebald gene plus a modifier. We mention the latter two incompletely understood spottings because we employed Berlin blaze in the experiments presently to be described.

In February, 1931, a mouse was discovered having a few white hairs on the forehead. Curiously enough, it bore no white on the belly, feet or tail. White spotting in some of these additional regions is found probably without exception in the head-blazed mice of all the previously described spotting varieties. The mutant was one of a litter of seven born in a cage where several females were mated to an unrelated male, but unfortunately the mouse was destroyed before its unique character was noted. Eighteen self young were recorded from this cage before October when there was

born a litter of four, three of which bore headdots. It may be supposed that one of the females although not spotted herself, carried a mutated gene in heterozygous state capable of marking a few of her offspring sired by a homozygous self male.

The mother of the headdots was detected and mated to her headdot son. A mouse with a large headdot was produced (Fig. 1, type 5) in addition to one having five white hairs, and also a self. Such results are expected if mother and son were both heterozygous for the headdot gene.

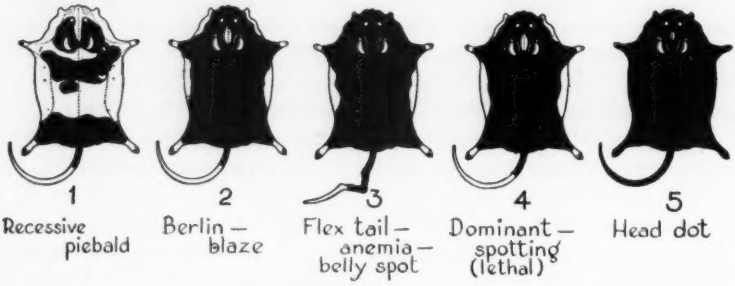

FIGURE 1

When a headdot stock had been produced by inbreeding, a series of crosses was made in which it was assumed that a mouse bearing a good headdot was homozygous and one bearing a few white hairs was heterozygous for the headdot gene. The results are shown in table 1. Headdots classed as homozygotes when mated *inter se* produced 80 headdots, 11 having a few white hairs, and 2 selfs. The results show that our original diagnosis of homozygous headdots was incorrect in a few cases or that homozygotes may frequently bear a few white hairs and occasionally may

TABLE 1

CLASSIFICATION OF THE PROGENY OF VARIOUS HEADDOT MATINGS

	HEADDOT	A FEW WHITE HAIRS	SELF
Headdot *inter se*	80	11	2
Headdot outcross	0	2	34
Headdot F_1	12	9	77
Headdot backcross	37	11 (1 with mottled tail tip)	33

be normal overlaps (selfs). Good headdots outcrossed to unrelated selfs produced 34 selfs and 2 bearing a few white hairs. The occurrence of the latter type indicates that heterozygotes may occasionally bear a few white hairs, as must have been the case with the first headdot discovered.

In an F_2 population there were 12 headdots, 9 bearing a few white hairs, and 77 selfs. Here we expect three times as many purebred selfs and heterozygotes (combined), as homozygous headdots. However, if we

assume that the 9 mice bearing a few white hairs are homozygous headdots, we still have fewer headdots than required by theory. Part of the deviation may be due to chance alone and part of it to normal overlaps. When supposedly homozygous headdots were mated to supposed heterozygotes there were produced 37 headdots, 11 bearing a few white hairs (one of which had a mottled tail tip), and 33 selfs. In this case equal numbers of homozygotes and heterozygotes are expected. It would hence appear probable that the 11 mice bearing a few white hairs included both homozygotes and heterozygotes. From these results it may be concluded that homozygous headdots usually bear a well developed white spot but may show only a few white hairs or may even be selfs, and that heterozygotes although usually selfs may occasionally develop a few white hairs. Very rarely mottling may appear upon the tail. This behavior shows that headdot is a distinct type of incompletely recessive spotting.

TABLE 2

CLASSIFICATION OF F_2 PROGENY OF THE CROSS OF HEADDOT WITH HAIRLESS

	BERLIN BLAZE		HEADDOT		A FEW WHITE HAIRS		SELF	
	NORMAL	HAIRLESS	NORMAL	HAIRLESS	NORMAL	HAIRLESS	NORMAL	HAIRLESS
All F_2 data	7	3	26	2	12	4	99	31
F_2 data excluding litters segregating for Berlin blaze	0	0	18	1	9	4	77	26
F_2 data excluding litters segregating for Berlin blaze, and those not segregating for headdot and hairless	0	0	11	1	2	4	30	14

The question arises whether headdot is an allelomorph of recessive piebald or due to an independent gene. If allelomorphic, it should be linked with recessive hairless, because piebald is linked with hairless. When Berlin blaze (modified piebald or a piebald allelomorph) was crossed with headdot most of the F_1 progeny were selfs but a few were headdots of low grade. This may be due to incomplete recessivity of the headdot gene if independent of Berlin blaze, or if allelomorphic to Berlin blaze, then it is due to dominance of the darker allelomorph as is the case when Berlin blaze is crossed to piebald. Hence the results of this cross allow us to draw no conclusions concerning the relationship between Berlin blaze and headdot.

Headdot females were mated to self hairless males. When their progeny were mated *inter se* they produced an F_2 population of 184 individuals classified in table 2. Seven were Berlin blaze, 3 Berlin blaze hairless; 26 were good headdots, 2 good headdot hairless; 12 bore a few white hairs, 4 bore a few white hairs and became hairless; 99 were selfs, and 31 were self hairless. The appearance of Berlin blaze was not surprising because the self hairless males employed in the P_1 were descended from a stock oc-

casionally segregating for Berlin blaze. The inclusion of litters bearing Berlin blaze animals may introduce an error into the study of linkage because we cannot tell whether or not an F_2 Berlin blaze segregate is homozygous for the headdot gene. Excluding litters in which Berlin blaze appeared the F_2 consisted of 18 headdots, 1 headdot hairless; 9 bearing a few white hairs, 4 bearing a few white hairs and hairlessness; 77 selfs, and 26 self hairless.

We may criticize these revised data because some of the matings did not segregate both headdot and hairless. This is important because it has been suggested that rarely a hairless mouse may be heterozygous for the ordinarily recessive hairless gene. Such a hairless mouse included in the P_1 might invalidate his descendants for the study of linkage. Likewise if one mistook a heterozygous headdot for a homozygote in the P_1 this introduces the possibility of unreliable linkage data from litters not segregating for headdot. When we exclude litters not segregating for hairless and headdot as well as those bearing Berlin blaze we find 11 headdot, 1 headdot hairless; 2 bearing a few white hairs, 4 bearing a few white hairs and hairlessness; 30 selfs and 14 both self and hairless.

It is evident in both original and revised data that hairless has not combined with headdot with quite so great a frequency as expected, whereas more selfs are hairless than expected in the case of genetic independence. If we exclude the class bearing a few white hairs as being of mixed genotypes we find the ratio of 30 self : 14 hairless : 11 headdot : 1 hairless headdot, where a ratio of 31.5 : 10.5 : 10.5 : 3.5 is expected with free segregation. If the deviation is not due to chance alone, then it suggests the repulsion phase of linkage with a crossover value of about 27 per cent. If hairless and headdot are linked it would appear likely that piebald and headdot are allelomorphs, but these data are too meager to be critical.

F_2 selfs from the linkage cross were mated *inter se*. They produced 6 Berlin blaze : 2 headdot : 1 bearing a few white hairs : 11 selfs. This shows that F_2 selfs may carry genes for Berlin blaze and headdot, but gives us no information upon the problem of allelomorphism. F_2 headdots mated to F_2 hairless gave 2 headdots : 3 bearing a few white hairs : 6 selfs : and 4 self hairless. This indicates that if linked a considerable number of crossovers occur because the headdot parent must have been heterozygous for hairless and the hairless parent heterozygous for headdot.

F_2 headdots were mated *inter se*. They produced 12 Berlin blaze, 3 Berlin blaze hairless; 18 headdots, 1 headdot hairless; 10 bearing a few white hairs; 8 selfs, 2 self hairless. But here again the interpretation is not clear. The fact that Berlin blaze is produced by supposedly homozygous headdots as well as the presence of selfs suggests that headdot and Berlin blaze are not allelomorphs and can be only weakly linked if linked at all. But these results may also be explained by hypothesizing allelo-

morphs and normal overlaps for the headdot character, although so many as 10 normal overlaps would hardly be expected in a population in which only 10 bearing a few white hairs were found.

However, if an F_2 Berlin blaze mouse can be shown to contain a gene for headdot then the possibility of allelomorphism is ruled out for such a mouse must bear 2 Berlin blaze genes plus 1 headdot gene whereas an animal can bear only two allelomorphic genes. An F_2 Berlin blaze female when mated to an F_2 self hairless male produced 2 Berlin blaze, 1 Berlin blaze hairless; 1 self hairless; 2 headdots. Likewise an F_2 Berlin blaze female mated to a purebred headdot male produced 2 headdots and 1 bearing a few white hairs. We may criticize these matings because the headdots which appeared might possibly be heterozygotes that received a headdot from the father only. Yet this seems unlikely because the 4 headdots could hardly all be due to incomplete recessivity of the headdot gene.

But there is a cross which will give a decisive answer to the problem. If an F_2 Berlin blaze is crossed to an unrelated self and their offspring mated *inter se*, then if headdots appear among the grandchildren it is because the original Berlin blaze mouse also carried the headdot gene. But if in this experiment some headdot progeny appear in the children this indicates that the F_2 Berlin blaze animal carried at least one headdot gene which is behaving as an incomplete recessive, and it is unnecessary to carry the experiment further. Two F_2 Berlin blaze females were crossed to a male of an inbred self stock. Together they produced 14 selfs and 3 bearing a headdot. The presence of headdots proves that their mother carried three spotting genes: two for Berlin blaze and one for headdot. Thus, headdot must be a new type of spotting independent of recessive piebald.

Conclusions.—Headdot is a new inherited type of spotting. It is irregular in its inheritance in that although it generally behaves as a recessive unit-character, it may fluctuate from a condition in which one gene for the character will produce a headdot to a condition in which it seems probable that two genes for the character fail to develop a headdot. This fluctuation is probably due at least in part to modifying genes. Headdot is not an allelomorph of recessive piebald.

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X CHROMOSOME INVERSIONS AND MEIOSIS IN DROSOPHILA MELANOGASTER

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It has long been obvious that inversions, like other chromosome aberrations, should be useful in studying the nature of meiosis. Previous studies of inversions in Drosophila have not led to a general theory of the behavior of inversions that is subject to quantitative tests. Our experiments together with the published cytological observations on plants have led to such a theory, outlined below. The detailed data on which our conclusions are based will be published elsewhere.

Single Exchange.—One of the reasons no general theory has been developed is that it has been inferred that single crossovers within the inverted section are rare (e.g., Sturtevant,[1] Gershenson[2]). The results now to be presented show that this conclusion is incorrect.

With regard to single exchanges between segments inverted relative to one another we know the following facts:

1. Single exchange probably occurs in all inversions with which we have worked. (See table 1 for list and description of these.) For long inversions the frequency of single exchange apparently approaches the frequency characteristic of the same segments normally arranged. The arguments in support of this conclusion follow.

We can regard Inv. scute-8 as practically a normal chromosome with the spindle attachment transposed from the right to the left end (table 1). From Inv. scute-8 over Inv. scute-4, a slightly shorter inversion, single crossovers are recovered and their frequency can be measured with fair accuracy. The observed frequency of recombinations is approximately normal; hence we can argue that Inv. scute-4 against a normal chromosome gives approximately the normal frequency of single crossovers but that they are not recovered. Inversion scute-8 over Inv. scute-7, a relatively short inversion, gives, in the common inverted segment, single crossovers which can be recovered by mating to males which give duplications and deficiencies of appropriate length. Here the frequency cannot be accurately measured but our data suggest that single exchange frequency is reduced in Inv. scute-7 to approximately one-half to two-thirds of the normal for the segment which is inverted. In a similar way we have demonstrated the occurrence of single crossovers in the common inverted segment of several other combinations of inversions although in many cases the relative inviability of the crossovers makes an accurate measure of their frequency difficult.

A second method that we have used involves the use of attached X females heterozygous for inversions. Such females heterozygous for Inv. yellow-4 give, by single exchange in the inversion, either (1) a closed X chromatid which can be recovered, or (2) a chromatid with two spindle attachments forming a chromatid tie between the egg nucleus and the second polar body nucleus. From the distortion of the sex ratio in the progeny it is inferred that the second result gives inviable eggs. The distortion of the sex ratio is therefore itself a measure of the frequency of single exchange. Both the number of recovered closed chromatids and the distortion of the sex ratio show that single crossovers occur with approximately normal frequency. Attached X females heterozygous for Inv. scute-7 give results as above, except that the closed chromatid cannot be recovered directly. Distortion of the sex ratio gives results in approximate agreement with those from Inv. scute-8/Inv. scute-7 discussed above. The sex ratio in $\widehat{XX}$ females heterozygous for Inv. delta-49 indicates that single crossovers within the inversion occur, but with a low frequency.

2. Single exchange must result in one chromatid with two spindle attachments and one with none, leaving two non-crossover normal chromatids. This is shown directly by cytological observations in Zea[3] and Trillium.[4] These cytological observations are in agreement with the genetic evidence given in the paragraph above in indicating that single crossovers occur within inverted segments.

3. Chromatids with two spindle attachments must form a tie between the first division nuclei, or between one pair of second division nuclei following a three-strand double, one exchange of which is inside, the other outside the inversion.

4. In Drosophila such a tie either does not break, or if it does the fragments are rarely or never included in the egg nucleus. This conclusion follows from the fact that duplication- or deficiency-carrying zygotes do not occur in the progeny of females heterozygous for inversions known to give single crossovers.

5. A single chromatid tie between first division nuclei does not result in the production of an inviable egg or a zygote inviable at a later stage. This conclusion comes from experiments in which egg and larval-pupal mortality were measured. Inversions known to give a high frequency of single crossovers give low egg mortality, and a mortality at later stages not higher than normal controls. Observed egg mortalities for certain inversions are as follows:

Inv. scute-8/+	6.8%
Inv. scute-4/+	7.5%
Inv. yellow-4/+	7.7%

If single exchanges gave rise to inviable eggs in half the cases, we should expect for these inversions approximately 50 per cent inviability.

The above facts, together with the fact that the second meiotic division spindles in the Drosophila egg lie with their axes approximately on the same straight line and form four nuclei likewise in a line, the inner one of which becomes the egg nucleus (Huettner[5]), lead us to propose the following scheme:

1. A single chromatid tie between two spindle attachments, one in each first division nucleus, prevents the tied daughter spindle attachments from going into the end nuclei but results in conditions (possibly mechanical orientation) such that the two normal chromatids always go to the end nuclei, one to each (Fig. 1). The chromatid with no spindle attachment goes to neither pole and is lost.

2. When both daughter spindle attachments of a chromosome in one first division nucleus are tied to spindle attachments in the other nucleus, nothing from the particular tetrad under consideration can go to the end nuclei during the second division (Fig. 1).

FIGURE 1

Above: the products of meiosis in the egg following a single exchange within a heterozygous inverted segment. Below: as above following a four-strand double exchange within the inverted segment. In both cases only the *X* chromosome tetrad is figured; each product, of course, contains one set of autosomes not shown in the diagrams. Either of the end products can be taken to represent the egg nucleus.

3. When the egg nucleus contains a spindle attachment with a chromatid tie to a spindle attachment in the second polar body nucleus, the egg is inviable, i.e., gives rise to a zygote which dies in the egg stage.

Applying the above hypotheses to the heterozygous inversion case, it is seen that: (1) Single exchange tetrads undergo reduction in such a way that only non-crossover chromatids are distributed to the end nuclei; the crossover chromatids are selectively eliminated. In half the cases of single exchange in the inversion accompanied by single exchange outside (three-strand doubles), one pair of second division nuclei will be connected by a chromatid tie,[4] which if it involves the inner nucleus, will result in an inviable egg. Except for the case of Inv. scute-7, lethal eggs from this source would be of negligible frequency in the inversions considered here. (2) Two-strand double exchange tetrads undergo reduction in the normal way; no chromatid ties are formed. (3) Three-strand double exchanges are equivalent mechanically to single exchanges but give rise to an egg nucleus

containing either a non-crossover or a double crossover chromatid. (4) Four-strand double exchanges give nothing but no-*X* nuclei. (5) Triple exchanges of the various possible types give results which can readily be worked out; the results of all of the types, occurring with frequencies determined by random exchange, together with a summary of the results from singles and doubles, are as follows:

EXCHANGE	NON-CROSSOVERS	DOUBLE CROSSOVERS	NO-*X* EGGS
None	1		
Single	1		
Double	3	3	2
Triple	7	21	4

The relations discussed below can be considered as tests of the above scheme.

1. There should be a quantitative relation between recovered double crossovers and patroclinous males. Inversions which give no double crossovers should give a low frequency of patroclinous males. (Normal females give some, presumably as the result of an independent mechanism.) With longer inversions the frequency of doubles increases and likewise the frequency of patroclinous males should increase. The frequencies of patroclinous males from several inversions are given in table 1.

TABLE 1

INVERSION	ENDS OF INVERSION (MAP POSITION)	PRIMARY EXCEPTIONS FEMALES %	PRIMARY EXCEPTIONS MALES %	SECONDARY EXCEPTIONS FEMALES %	SECONDARY EXCEPTIONS MALES %
+/+ (standard control)		0.02	0.08	4.3	4.3
Scute-7/+	0+ to 14±	0.09	0.28	12.9	15.1
Delta-49/+	11± to 42±	0.00	0.20	45.4	41.9
ClB/+	6± to 59.4	0.25	0.47	36.6	37.8
Bobbed-df/+	9± to 64±	0.00	1.95	13.7	15.9
Yellow-4/+	0− to 61±	0.05	2.34	6.8	8.9
Scute-4/+	0+ to 64±	0.02	5.75	4.3	13.4
Scute-8/+	0+ to 66	0.02	3.20	9.9	13.8
Scute-4/delta-49		0.00	0.17		
Scute-8/delta-49		0.26	0.34	47.3	43.5
ClB/delta-49		0.32	0.16		
Delta-49/yellow-4		0.32	0.13	53.2	53.2

Scute-7 inversion and Inv. delta-49 give few or no doubles; patroclinous males are very low in frequency. ClB inversion gives recovered doubles with a low frequency; patroclinous males are low but higher than from Inv. scute-7 or Inv. delta-49. The other inversions give appreciable frequencies of doubles. The relation between doubles and patroclinous males from experiments where practically all doubles could be detected in the males and where, at the same time, patroclinous males could be detected, are as follows:

CONSTITUTION	REGULAR MALES	CROSSOVER MALES	PATROCLINOUS MALES
Bobbed-df/+	3261	93	66
Yellow-4/+	2063	57	51
Scute-4/+	1090	108	63
Scute-8/+	1677	93	57
Scute-8df/+ (lethal in males—numbers corrected)	422	60	31
Total		411	268

From double exchanges we expect 3 double crossover males to 2 patroclinous males. None of the counts shows significant deviations from this ratio and the total gives a ratio of 3.22 to 2, a deviation from 3:2 not statistically significant. From the longer inversions we expect a low frequency of triple exchanges which would change the ratios to 3 +:2. Actually the inversions giving the highest frequencies of crossovers show the greatest deviation from a 3:2 ratio; these deviations are in the expected direction.

2. Reducing crossing-over in a long inversion by putting it over a shorter one should decrease the frequency of patroclinous males as well as of double crossovers. We know that, from the combinations given in table 1, no double crossovers are recovered. Patroclinous males are very low in frequency.

3. The frequency of exceptional females should be as low as that from normal females and should not vary with crossing-over. From table 1 it is seen that this expectation is approximately realized.

4. The frequency of inviable eggs from females carrying an inversion should be equal to the frequency of patroclinous males (no-X eggs fertilized by Y and X sperms should account for these two classes). The egg mortality data from inversions considered in relation to more or less comparable normal controls (strictly comparable controls cannot be had), show that this is so.

It is clear, then, that the hypothesis is in reasonably good agreement with the available evidence from heterozygous inversions.

There is another test which can be applied, namely, the behavior of heterozygotes and homozygotes for a closed X chromosome. These have been studied by L. V. Morgan.[6] Comparing the published data with expectation according to the above scheme, we find two important discrepancies: (*a*) The non-crossover classes from X/X^c are not equal, there being an excess of X over X^c individuals. (*b*) The inviable eggs from X/X^c are much higher in relation to doubles than expected. Experiments using a second closed X chromosome found by Mr. R. D. Boche of this laboratory show that both of these discrepancies are due to viability effects. Data from both heterozygotes and homozygotes for this new closed X chromosome will be considered in detail in another publication, but we can say here that all of the data are in substantial agreement with the scheme under consideration.

Secondary Non-Disjunction.—Heterozygous inversions give secondary non-disjunction with a frequency which shows a very strong negative correlation with crossing-over. This can readily be seen to be true in table 1. Inversions ClB and delta-49 which show low crossing-over for the whole chromosome give high frequencies of secondary exceptions. Combinations of long against short inversions likewise give high frequencies. Since *XXX* and *YO* zygotes are lost the frequency of exceptional gametes will differ from that of exceptional zygotes; where q is taken as the proportion of exceptional zygotes and p the proportion of exceptional gametes, $p = \frac{2q}{1+q}$. From the data given in table 1 and from additional data not included here, p appears to approach 0.667 as a maximum. Since the $X-Y$ synapsis scheme proposed by Bridges[7] gives a maximum p value of 0.5, clearly below that indicated by the inversion data, it becomes necessary to search for a new interpretation of secondary non-disjunction. We know that secondary exceptional gametes practically always come from non-crossover X tetrads. Such tetrads in the presence of a Y chromosome evidently undergo a preferential segregation similar to that studied by Sturtevant[8] for the fourth chromosome. Non-crossover X tetrads plus a Y must give a higher frequency of XX-Y than X-XY separation. The data are inadequate to measure accurately the extent of this preference. The constant value 0.667 (limiting value for certain types of fourth chromosome arrangements) gives results that are not unreasonable. Additional experimental data are needed.

General Conclusions.—It has been shown above that crossovers between X chromosome segments inverted with respect to one another influence the orientation of the meiotic tetrads in such a way as to eliminate the single crossover chromatids, leaving a normal non-crossover one in the reduced egg nucleus. The more complex results expected from multiple exchange are analyzed and shown to give results in agreement with observation. It seems probable that the scheme outlined in this paper will apply for the case of inversions that do not include the locus of the spindle attachment and for other chromosome aberrations in which crossing-over gives rise to chromatids with two spindle attachments, and where the conditions of meiosis are such that (*a*) the meiotic spindles are oriented so that the reduced nuclei lie approximately on a single straight line, and (*b*) only one of the terminal nuclei functions in further development. These conditions obtain in megasporogenesis in most seed plants, as well as in oögenesis in most animals.

Discussion of the bearing of this scheme on the mechanism of normal disjunction, on the evolutionary significance of inversions, and similar elaborations, is omitted for lack of space.

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LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS)

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The mutated genes of the rat now on record are as follows:

A, a	agouti, non-agouti
B, b	black, chocolate
C, c^r, c	fully colored, ruby-eyed, true albino
Cu, cu	curly, normal coat
Cu_2, cu_2	curly, normal coat
D, d	intense, dilute
H, h^I, h	self, Irish, hooded
Hr, hr	normal coat, hairless
K, k	normal coat, kinky
P, p	fully colored, pink-eyed yellow
R, r	fully colored, red-eyed yellow

Of these *A*, *C* and *H* were found by Castle to be independent of each other, but *P*, *R* and *C* were found by Castle and Wright, Dunn and Wachter to lie in a common linkage system. Subsequently Roberts discovered genes *d* and *hr* and showed that they are probably independent of the genes previously known (*A C H P* and *R*). More recently King has discovered the dominant mutation, curly (*Cu*), and the recessive mutation, brown or chocolate (*b*). Linkage studies on these newly discovered genes will be reported in this paper. Gregory has recently described a second dominant curly mutation apparently distinct from the curly described by King; and Feldman (unpublished data) has found a recessive mutation called ***kinky***, which even more strongly than the "curly" mutations has a tendency to shorten the hair and make it curly. We are indebted to Dr. Feldman for an opportunity to study the kinky mutation in advance of his forthcoming publication concerning it.

The increasing number of mutated genes increases the probability that additional genetic linkages will be discovered, if search is made for them,

since the haploid number of chromosomes of the rat is known to be twenty-one, and at least five of these are already tagged with identifying genes, viz., (1) *A*, (2) *CPR*, (3) *D*, (4) *H* and (5) *Hr*. The required experimental matings have been made by King, while Castle has coöperated in the genetic analysis of the experimental data and has written this report.

1. First we may report an experiment to discover whether the new genes, brown and curly, are linked with each other. A cross was made between cinnamon females (*b cu*) and curly males (*B Cu*). The F_1 animals were gray and curly (*Bb Cu cu*). A backcross was made of the F_1 animals with the cinnamon parent race, the double recessive combination. It is expected that, if there is no linkage, four classes of offspring will be produced, all equally numerous. The observed result is as follows:

Gray curly (*B Cu*)	Gray straight (*B cu*)	Cinnamon curly (*b Cu*)	Cinnamon straight (*b cu*)
190	125	128	182

The two middle groups are the crossover classes. They are both smaller than the non-crossover classes. Together they number 253, whereas the non-crossovers total 372. This is a clear indication of linkage, for we expect half of the young to be crossovers if there is no linkage. The observed departure from expectation is 59.5 ± 8.43, a deviation clearly significant, since it is more than 7 times the probable error. A similar indication of linkage is obtained irrespective of whether the F_1 parent is a male or a female as is shown in table 1.

TABLE 1

CLASSIFICATION OF THE YOUNG PRODUCED IN A TEST FOR LINKAGE BETWEEN CURLY AND BROWN

	GRAY CURLY	GRAY STRAIGHT	CINNAMON CURLY	CINNAMON STRAIGHT	TOTAL	PER CENT CROSSOVERS
Hybrid females backcrossed to cinnamon males	122	67	76	106	371	38.54 ± 1.75
Hybrid males backcrossed to cinnamon females	68	58	52	76	254	43.30 ± 2.12
Total	190	125	128	182	625	40.48 ± 1.35

In certain experiments with rats and mice, it has been found that crossing-over occurs more frequently in females than in males, but the present experiment lends no support to this idea, as is shown by table 1. Linkage between curly and brown is shown in both sorts of backcross matings, but the indicated crossover percentage is actually greater in the case of hybrid males than in the case of hybrid females, though on account of smaller numbers, the probable error is greater. Nevertheless this discordant result

rests on too slender a statistical basis to disprove the generalization drawn by Castle and Wachter from very extensive observations.

2. A mating between curly males and inbred albino females affords tests for linkage between curly (*Cu*) and the three genes *a*, *c* and *h*. The parents were, respectively, *A C Cu H* and *a c cu h* in formula. Consequently the F_1 animals were heterozygous for all four genes. Since the albino stock was homozygous for the four recessive genes, a backcross was made to this race. A population of 136 backcross young has been recorded, 72 individuals being curly and 64 straight-haired, expectation being 68 ± 3.9 of each. The ten expected phenotypes are all present but not exactly in the expected proportions, the hooded classes being small, possibly because of the classification of certain wide striped hooded animals as non-hooded. Nevertheless the relation of curly to each of the other genes (including *h*) shows that no linkage is involved.

The ten phenotypes recorded, and their frequencies, were as follows:

Albino curly	28
Albino straight	21
Gray curly	19
Gray straight	20
Black curly	16
Black straight	15
Gray hooded curly	7
Gray hooded straight	2
Black hooded curly	2
Black hooded straight	6
Total	136

The relation of curly to each of the genes, *A*, *C* and *H* can be shown as in table 2.

Curly, in relation to albinism, shows slightly more crossovers (the middle classes) than non-crossovers (the outside classes). Hence there is no indication of linkage in that category. In relation to hooding, crossovers

TABLE 2

RECOMBINATIONS IN THE BACKCROSS

A Cu	*A cu*	*a Cu*	*a cu*	CROSSOVERS	NON-CROSSOVERS
26	22	18	21	40	47
C Cu	*C cu*	*c Cu*	*c cu*		
44	43	28	21	71	65
H Cu	*H cu*	*h Cu*	*h cu*		
35	35	9	8	44	43

and non-crossovers are as nearly equal as possible. In relation to agouti, non-crossovers are in excess. This would suggest linkage if the deviation

were of significant magnitude. But it is only 3.5 ± 3.1. In other words, it is scarcely greater than the probable error and so no greater than random sampling might cause.

3. A cross between cinnamon males and albino females produced F_1 gray offspring which were bred *inter se* to produce an F_2 population consisting of 121 individuals. The parents were, in formula, *A b c H* and *a B c h*, respectively, and the F_1 individuals consequently would be heterozygous for all four differential genes. If there is no linkage between any of these, we expect classes to result, with the frequencies indicated, for nine different phenotypes, as follows:

	OBSERVED	EXPECTED
Albino	34	30.2
Gray	38	36.7
Cinnamon	11	12.2
Black	14	12.2
Brown	8	4.0
Gray hooded	8	12.2
Cinnamon hooded	4	4.0
Black hooded	3	4.0
Brown hooded	1	1.3
Total, colored	87	86.6

Agreement between observed and expected numbers is excellent, making it clear that no linkage exists between gene *B* and any one of the three genes *A*, *C* and *H*. For, if we consider the three cases separately, it will be seen that if linkage (repulsion) existed between albinism and brown, there should be an excess of brown pigmented individuals among the colored. The number expected if no linkage occurs is 21.75, the number recorded is 24, an insignificant excess of 2.25, the probable error being 2.72. If linkage existed between agouti and brown, we should expect an excess of cinnamons and a deficiency of browns. Instead, we observe the opposite, a slight deficiency of cinnamons (15 where 16.2 are expected) and an excess of browns (9 where 5.3 are expected). If linkage existed between brown and hooding, we should expect a deficiency of brown hooded and an excess of browns (not-hooded). We have recorded 5 brown hooded where the expectation is 5.44, and 19 browns (not-hooded) where the expectation is 16.2. These deviations are without statistical significance, the excess of browns being only 2.8 where the probable error is 2.46.

Accordingly, we find no indication of linkage between the brown gene and either of the three genes, *A*, *C* and *H*. This supports the conclusions derived from the curly $\times$ albino cross, for if curly is linked with brown, all genes which show no linkage with curly should also show no linkage with brown.

4. In order to make a test for linkage between curly and dilution, curly coated individuals were crossed with blues. The F_1 animals were then backcrossed with double recessives, blue straight. A population of 312 young was thus obtained, distributed as follows:

Intense curly	Intense straight	Dilute curly	Dilute straight
71	91	65	85

The crossover classes, intense straight and dilute curly, together number 156. The non-crossover classes, intense curly and dilute straight, also number 156, indicating that no linkage exists.

5. In March, 1934, we received from Dr. H. W. Feldman, of the University of Michigan, some stock of a new rat mutation resembling curly, but unlike it, being a recessive rather than a dominant in heredity. An exchange of animals between our laboratories had been arranged in order that breeding tests might be made to ascertain whether the mutation discovered by Feldman was (1) an allelomorph of curly or (2) linked with it or (3) wholly free from it. The tests which have now been made show that the last named hypothesis is the correct one. Kinky (as the Feldman mutation will hereafter be called, to distinguish it from curly, which it closely resembles phenotypically) does not lie in the same chromosome as curly and cinnamon.

A cross was made between a black hooded kinky male (*aa hh kk*) received from Dr. Feldman and gray curly coated females (*AA Cu Cu*). The F_1 young were all gray coated but bore a white belly spot, indicating that they were heterozygous for the hooded pattern. Their genetic formula accordingly would be *Aa Cu cu Kk Hh*. They were backcrossed reciprocally to black kinky mates heterozygous for hooding (*aa kk Hh*). The groups of young produced by the reciprocal backcrosses were not qualitatively different, hence there is no indication of sex linkage, and the two series may be combined (see table 3).

TABLE 3

RESULTS OF RECIPROCALLY BACKCROSSING (TO THE BLACK KINKY PARENT RACE) F_1 HYBRIDS BETWEEN GRAY CURLY FEMALES AND A BLACK HOODED KINKY MALE

TYPES OF YOUNG	F_1 ♀♀ × *aakk* ♂	F_1 ♂ × *aakk* ♀♀	TOTALS
Gray curly	47	30	77
Gray straight	19	6	25
Gray hooded curly	5	4	9
Gray hooded straight	1	1	2
Black curly	48	25	73
Black straight	18	9	27
Black hooded curly	8	1	9
Black hooded straight	1	1	2
Totals	147	77	224

Since it was known from experiments previously made by Dr. Feldman that kinky is a recessive character, it is certain that the curly character seen in the F_1 young was a manifestation of the curly gene inherited from their curly parent, for the kinky gene would find no expression in the F_1 individuals. Nevertheless they would all be heterozygous for that gene as well as for the curly gene. (1) If *Cu* and *k* were allelomorphs, the gametes formed by the F_1 parent would be either *Cu* or *k*, and in a backcross with *kk* animals, all young should be curly coated. In reality 168 were curly and 56 straight, hence *Cu* and *k* are not allelomorphs. (2) If *Cu* and *k* were borne in the same chromosome, less than half the gametes formed should be crossovers (*Cu k* or *O*). It would be impossible to detect in the backcross the presence of a gamete *Cu k*, since it would produce the curly type of coat, like *Cu* and *k* singly. But the *O* combination (lacking both *Cu* and *k*) should produce normal (straight coated) offspring, since there would be in the zygote only a single *k* gene derived from the kinky parent. Accordingly linkage would be indicated in the backcross by the production of straight coated young *significantly fewer than* 25 per cent of the population. The population raised consists of 224 individuals, of which exactly one-fourth (56) were straight coated. This is conclusive evidence that *Cu* and *k* are independent, lie in different chromosomes.

Genes *A* and *k* also entered this cross in the repulsion relationship. If they were linked, the crossover combinations *ak* and *AK* should be deficient in the backcross, that is, should represent significantly less than half the population. The four types gray curly, gray straight, black curly and black straight occurred in the respective frequencies 86:27:82:29 where the ratio expected if no linkage exists is 3:1:3:1. The second and third of these are crossover combinations, their sum being 109, the noncrossovers totaling 115. The deviation from the expected equality is 3 ± 5.05; hence less than might be expected to occur by random sampling alone. Accordingly we may conclude that no linkage exists between the genes *A* and *k*.

Genes *h* and *k* entered this cross in the coupling relationship, and should show association significantly oftener than dissociation, if linkage existed between them. In reality 22 hooded young were recorded in the backcross, of which 18 were curly and 4 straight, expectation being 5.5 straight, if no linkage occurs. The departure from expectation is 1.5 ± 1.58, in other words, less than random sampling alone might produce. Hence there is every reason to believe that *h* and *k* segregate independently and lie in different chromosomes.

It is therefore clear, from the experiment described, that the kinky gene does not lie in the chromosomes tagged by the genes *A*, *Cu* and *H*. If it does not lie in the *Cu* chromosome, it should show no linkage with the *b* gene, which we have shown to be linked with *Cu*.

6. The relationship of gene *k* to *b* (and certain other genes) was tested in a mating between cinnamon females homozygous for the *A* gene (*AA bb*) and the same black hooded kinky male (*aa hh kk*) which was employed in the cross with curly females. The F_1 gray animals from this cross were thus heterozygous for four genes (*Aa Bb Hh Kk*) and would be expected to form 16 classes of gametes, all equally numerous, if no linkage exists between any of the four genes. The backcross was made reciprocally to cinnamon kinky individuals obtained as representatives of the double recessive combination (*bb kk*) in the F_2 generation. They were also heterozygous for *A*, and some of them for *H* also. The very fact that the double recessive combination (*bb kk*) was readily obtained in F_2 indicates that no

TABLE 4

RESULTS OF RECIPROCALLY BACKCROSSING (TO CINNAMON KINKY) F_1 INDIVIDUALS FROM A CROSS BETWEEN CINNAMON FEMALES AND A BLACK HOODED KINKY MALE

TYPES OF YOUNG	F_1 ♀♀ × CINNAMON KINKY ♂♂	F_1 ♂♂ × CINNAMON KINKY ♀♀	TOTALS	APPROXIMATE EXPECTATION
Gray straight	19	18	37	40.5
Gray kinky	25	24	49	40.5
Gray hooded straight	5	6	11	13.5
Gray hooded kinky	3	9	12	13.5
Cinnamon straight	25	23	48	40.5
Cinnamon kinky	20	24	44	40.5
Cinnamon hooded straight	10	3	13	13.5
Cinnamon hooded kinky	3	5	8	13.5
Black straight	5	5	10	13.5
Black kinky	6	8	14	13.5
Black hooded straight	1	1	2	4.5
Black hooded kinky	1	0	1	4.5
Brown straight	4	1	5	4.5
Brown kinky	2	9	11	4.5
Brown hooded straight	1	3	4	1.5
Brown hooded kinky	0	1	1	1.5
Totals			270	264.0

linkage exists between *B* and *K;* nevertheless it was thought desirable to obtain demonstrative evidence by carrying out the backcross matings. Fifteen of the expected 16 phenotypes were obtained in each of the reciprocal backcrosses, a surprisingly close agreement with expectation, in view of the fact that the total number of young in one backcross was 130 and in the other 140. Combining the results of the reciprocal backcrosses, which show no qualitative difference and thus give an emphatic negative to the existence of sex linkage on the part of either gene, we have all 16 phenotypes represented by one or more individuals in a total of 270 young. The most probable expectations are shown in the last column of table 4. With these the observed frequencies agree excellently.

As a test for linkage between the genes *A* and *k*, the data contained in table 4 may be arranged as follows. These genes entered the cross in the repulsion relationship and should recombine freely, if no linkage exists, so as to produce four phenotypes (other differences being disregarded).

	AK	*Ak*	*aK*	*ak*
Observed	109	113	21	27

If all the cinnamon kinky parents had been heterozygous for *A*, we should expect the four classes to be as 3:3:1:1, but it is probable that some of them were homozygous for *A*, hence the deficiency of the non-agouti classes below that expectation. Linkage would result in making the *AK* and *ak* classes, which are crossovers, less than the non-crossover classes, *Ak* and *aK*. The sum of the former is 136, that of the latter 134. Hence no linkage is indicated between *A* and *k*.

As a test for linkage between the genes *B* and *K*, the data contained in table 4 may be grouped thus:

	BK	*Bk*	*bK*	*bk*
Observed	60	76	70	64

The crossover classes (*BK* and *bk*) together include 124 individuals, the non-crossovers (*Bk* and *bK*) number 146. The deviation from equality of the two groups is 11 ± 5.54, which is statistically without significance.

As a test for linkage between *H* and *K*, the data contained in table 4 may be grouped thus:

	HK	*Hk*	*hK*	*hk*
Observed	100	118	30	22

The number of hooded individuals recorded is possibly less than it should be, for the reason stated elsewhere, but the proportion of kinkys among those classified as hooded is pertinent to the question of linkage. There are 30 hooded straight to 22 hooded kinky, where equality is expected if no linkage occurs. The departure from equality is 4 ± 2.86, which is without statistical significance. Further, the crossover classes (*HK* and *hk*) together number 122 individuals, the non-crossovers (*Hk* and *hK*) number 148. The deviation from equality is here 13 ± 5.54, which again is devoid of statistical significance.

Accordingly, we find that the crosses of kinky with curly and kinky with cinnamon, as recorded in tables 3 and 4, agree in showing no linkage to exist between gene *k* and the genes *A* and *H*. Further table 3 has shown the gene *Cu* to be free from *K*, and table 4 has shown *B* to be free from *K*. These several findings are all compatible with the previously demonstrated linkage between *B* and *Cu*.

7. A report may be made also on a test for linkage between curly and hairless, the results of which indicate free assortment of these genes. A

gray curly female was mated to a pink-eyed yellow hairless male, producing a litter of six gray curly females. These were backcrossed to the yellow hairless male, their father, or to hairless males of similar (double recessive) constitution. Disregarding the segregation for coat color, we may classify the backcross young thus:

Curly normal	Curly hairless	Straight normal	Straight hairless
28	18	24	15

Fewer hairless than normal-haired young were recorded, doubtless owing to the inferior survival ability of hairless individuals; but in relation to curly, hairless obviously segregates freely. The crossover classes, curly hairless and straight normal, together number 42: the non-crossover classes number 43, a result as close to equality as possible in an odd number of young.

The gene for yellow lies in the same chromosome as albinism. The results of this cross show that yellow is independent of curly, thus supporting the result of crosses 2 and 3, which showed albinism to be independent of curly and brown, for the 85 backcross young produced in this experiment may be grouped thus:

Gray curly	Gray straight	Yellow curly	Yellow straight
15	17	31	22

The sum of the crossover classes, gray straight and yellow curly, is 48, exceeding that of the non-crossover classes, 37. Consequently no linkage is indicated.

This same cross shows that hairless is independent of yellow (and so of albinism), confirming the conclusion of Roberts. The 85 backcross young may be grouped thus:

Gray normal	Gray hairless	Yellow normal	Yellow hairless
20	12	32	21

The crossover classes, gray hairless and yellow normal, aggregate 44; the sum of the non-crossover classes is 41. Clearly no linkage exists between yellow and hairless.

Summary.—Linkage studies show that in the common rat, in which eleven genes have been identified, there occur two linkage systems, one containing the three genes *C*, *P* and *R*; the other, the two genes *B* and *Cu*. The crossover percentage between *B* and *Cu* is 40.48 ± 1.35, which indicates that these two genes lie rather far apart in the same chromosome. They recombine independently of the genes *A*, *C*, *D*, *H*, *Hr* and *K*, and so must lie in a different (sixth) chromosome. It remains to canvass their linkage relations with the gene for curly No. 2.

Incidentally it is shown that the gene *K* does not lie in the same chromosome as *A* or *H*. Its independence is thus established as regards three of

the six chromosomes mentioned, but its relation to the chromosomes which carry genes *C*, *D* and *Hr* is as yet unknown.

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X-RAY AND ABNORMALITIES: INCREASED ABNORMALITY OF SEGMENTS IN DROSOPHILA DUE TO X-RAYING OF GAMETES

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The study of genetic effects of x-ray can be considered a field rich in resources clarifying much as to the behavior and effect of gene and chromosome constitution. Morgan, Muller,[1] Bridges,[2] Patterson,[3] Painter, Dobzhansky, Schultz and others who worked with Drosophila have already offered explanations of many genetic phenomena. Mutations of genes, translocations and eliminations of chromosome parts with corresponding phenotypic, genetic, lethal and semi-lethal effects all due to x-ray has stimulated interest in this type of research the world over. Some work of this nature was attempted here during the period December, 1933 to September, 1934.

The favorite of genetic workers, Drosophila melanogaster, was chosen as subject, not only because of the simplicity of handling, relatively short life cycle and well-known chromosome composition, but also because reports on similar previous experiments are available.

The stock chosen was the normal unmarked Iowa wild (a stock bred in the Iowa laboratories since June, 1931 from captured Drosophila). To simplify breeding, the experimental results were limited to the consideration of changes on the sex chromosome. To secure results in shortest time, virgin females were x-rayed, and mated, so that any changes produced in the *X*-chromosome would show up in the males of the first generation. Group I, twenty-nine females, was given a dosage of 731 r units. (With the x-ray machine used this was 50 kv., 5 m.a., target distance of 12 cm., Al filter of 1 mm. for 20 min.) Group II, twenty-four females, was given 1462 r units. (Other conditions remaining the same, rayed for 48 min.) Twenty-nine females (Group III) were used as controls for both

x-rayed groups. Two thousand nine hundred thirty F_1 offspring of the first group were examined, 2615 F_1 of the second and 2949 F_1 of the controls

TABLE 1

FREQUENCY OF UNUSUAL CHARACTERISTICS IN F_1 OF CONTROL AND X-RAYED INDIVIDUALS

	CONTROL ♀s		X-RAYED ♀s 731 r		X-RAYED ♀s 1462 r		CONTROL ♂s		X-RAYED ♂s 1462 r		X-RAYED ♂s 3700 r	
	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s
Number of F_1 examined	1447	1502	1472	1458	1249	1366	96	90	91	63	34	29
Number with unusual wing characters	18	8	5	6	9	8	1	3	3	1	1	
% with unusual wing characters	0.882		0.375		0.650		2.151		2.600		1.587	
Number with pupa case clinging to thorax	1		15	2	6	3	7	3	3	4	3	2
% with pupa case clinging to thorax	0.034		0.580		0.306		5.37		4.54		7.94	
Number with abnormal abdomen	19	10	17	14	40	39	1	1	3	1	1	1
% with abnormal abdomen	0.983		1.058		3.021		1.08		2.6		3.175	

(table 1). Both males and females having unusual wing characteristics (such as spread wings, raised wings, broken or notched wings and balloon-like expansions on the wings), pupa case clinging to back of thorax were noted, but breeding tests failed to show that these were heritable or that they varied from x-rayed to control groups.

The most remarkable change shown in this experiment was the appearance of irregular abdominal segments of both males and females. In many cases entire segments might be missing, or right, left or median dorsal half of segments. In some cases this produced peculiarly rotated abdomens.

Of the F_1 of controls (table 4) 0.983% had irregular abdomens, of Group I (irradiated with 731 r), 1.058% and of Group II (irradiated with 1462 r), 3.021%. In the F_1 of controls the irregularity affected only one segment in 83.2% of the individuals having irregular abdomens and produced no rotated abdomens. Of the irregularities appearing in Group I, 74.2% had one segment affected, but 25.8% had two or more segments irregular or had rotated abdomens. In Group II, 71.8% had one segment irregular and 28.2% had two or more segments irregular with rotated abdomens. It would seem from these data that x-raying in some way affected the development of the segments and that the effect was greater with increase of irradiation.

Morgan[4] reports the gene for abnormal abdomen as being a dominant on the first chromosome. Its effect is very irregular in its appearance,

being most prevalent among the first offspring developing in a moist abundant food supply and gradually dwindling to an insignificant amount as the culture becomes dry and depleted. No tests were made to trace the genetic solution of the irregular abdomen which appeared in the recent experiments at Iowa. It was only noted that the second and third generations from those with peculiar abdomens had approximately the same per cent irregularities as the control group (table 2).

TABLE 2

FREQUENCY OF ABNORMAL ABDOMENS IN F_2 AND F_3 INDIVIDUALS FROM F_1 OF ORIGINAL GROUPS HAVING ABNORMAL ABDOMENS

	CONTROL ♀s		X-RAYED ♀s 731 r		X-RAYED ♀s 1462 r		CONTROLS ♂s		X-RAYED ♂s 1462 r		X-RAYED ♂s 3700 r	
	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s	♀s	♂s
Number of F_2 examined	361	398	963	974	765	807	42	35	12	14	27	22
Number F_2 with abnormal abdomen	3	7	11	8	15	8	1	0	0	0	0	1
% of F_2 with abnormal abdomen	1.32		0.98		1.463		1.3		0		2.041	
Number F_3 examined	408	398	763	717	555	568						
Number F_3 with abnormal abdomen	10	9	12	13	17	12						
% of F_3 with abnormal abdomen	2.357		1.689		2.582							

Since it was unfertilized eggs that were x-rayed and abnormal abdomens appeared in both sons and daughters of females bearing these eggs and the characteristic did not appear in genetic proportions, it would seem that the change was extra-genic. Furthermore, had the rays affected the chromosome to produce a translocation or an elimination of a portion of the chromosome, the effect would be visible in future generations. Since such hypotheses are not supported by the data obtained (tables 1 and 2), further estimations must be made in which a permanent chromosome change does not take place.

Muller[5] has offered an explanation in this connection that x-rays might affect the germ cells after a period of division in preparation for later mitoses. If the chromosome strands had undergone division previous to x-raying, perhaps only one strand would be affected (fractional effect). This would produce mosaic individuals and only if any of their germ cells contained affected chromosome strands would the offspring show this character. If the x-raying altered the mitotic mechanism, the cell division might be irregular and produce unusual and asymmetric segments. If any of the above suppositions are correct, the same results would be expected in the offspring of x-rayed males as of females.

However, the hypothesis could be advanced that the x-rays might have affected the cytoplasm in such a way as to cause the unusual sclerites.

Just[6] has presented arguments proposing the primary effect of x-ray to be on the cytoplasm of the germ cells. He concludes "that the injury to the cytoplasm is cortical injury. Normal chromosome distribution and combination depend upon the integrity of the cortex; their aberrant behavior is the effect of the loss of this integrity." Since the cytoplasm may affect the genes in but one individual temporarily, the abnormal abdomen would not appear in the offspring. Whether or not the stock contained any genes for abnormal abdomen and the x-ray amplified their effect is not known. However, it is known that irregular abdomens appeared in the controls, but in greater proportion and irregularity in the x-rayed groups. Since the cytoplasm of the sperm is a minimum, so

TABLE 3

FREQUENCY OF MALES AND FEMALES OF EXPERIMENTS GIVING OFFSPRING WITH ABNORMAL ABDOMENS

	CONTROL ♀s	X-RAYED ♀s 731 r	X-RAYED ♀s 1462 r	CONTROL ♂s	X-RAYED ♂s 1462 r	X-RAYED ♂s 3700 r
Number in experiment	29	29	24	6	6	8
Number giving offspring	24	23	23	4	5	6
Number giving no offspring with abnormal abdomen	12	10	9	2	3	3
% giving no offspring with abnormal abdomen	50	43	39	50	60	50
Number giving one offspring with abnormal abdomen	3	4	2	2	1	2
% giving one offspring with abnormal abdomen	13	17	8.7	50	20	33
Number giving two offspring with abnormal abdomen	5	4	4			1
% giving two offspring with abnormal abdomen	21	17	17			17
Number giving 3 or more offspring with abnormal abdomens	4	5	8		1	
% giving 3 or more offspring with abnormal abdomens	17	22	35		20	

also would we expect its changes to be. Substantiation of this hypothesis of the effect of x-rayed cytoplasm and the disproving of the other theories is suggested in another experiment that was attempted in which males were x-rayed.

For this, Morgan "+" (1912) stock was used. Two generations preceding the subjects were examined and those individuals having unusual abdomens were eliminated. The rate of appearance remained the same, being 0.85% and 1.3% for the first and second generations, respectively. The subjects were irradiated in two groups with 1462 r (to check previous

experiment, but using another x-ray machine operated at 50 kv., 5 m.a., target distance of 19.05 cm., Al filter 1 mm., for 67 minutes) and 3700 r (other conditions remaining the same, rayed 2 hr. 51 min.). All F_1 were examined and the per cent of appearance of abnormal abdomens was 1.08 in controls, 2.6 in those irradiated 1462 r and 3.175 for those given 3700 *with no markedly rotated abdomens.* These data are based on counts of 186, 154 and 63 F_1 flies and cannot be considered significant in comparison with those of the first experiment which involved thousands of flies. But in proportion to the radiation the percentages are not as great as those from x-rayed females, though the percentage of irregular abdomens in controls can be considered the same (table 1). Breeding tests did not

TABLE 4

DEGREE OF IRREGULARITY AMONG INDIVIDUALS HAVING ABNORMAL ABDOMENS

	TOTAL % WITH ABNORMAL ABDOMENS	% OF THOSE WITH ABNORMAL ABDOMENS HAVING ONE SEGMENT AFFECTED	% OF THOSE WITH ABNORMAL ABDOMENS HAVING TWO OR MORE SEGMENTS AFFECTED
F_1 of control	0.983	83.2	16.76
F_1 of x-rayed 731 r	1.058	74.2	25.8
F_1 of x-rayed 1462 r	3.021	71.8	28.2

show this to be definitely inherited. It seems reasonable to conclude, with respect to this case of abnormal abdomens that when corresponding x-ray treatments of germ cells of male and female Drosophila produce different results, the explanation of the phenomena must be sought elsewhere than in chromosome activities which are similar in both sexes.

It may be concluded that x-ray can affect the cytoplasm of a gamete in such a way that the individual developing from it shows the change. The character, however, is not transmitted to the offspring. Since the egg contains a great deal more cytoplasm than the sperm, it is to be expected that the offspring of x-rayed females would show greater changes than those of x-rayed males.

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THE ANCESTRAL TREE OF THE PROBOSCIDEA. DISCOVERY, EVOLUTION, MIGRATION AND EXTINCTION OVER A 50,000,000 YEAR PERIOD[1]

BY HENRY FAIRFIELD OSBORN

THE AMERICAN MUSEUM OF NATURAL HISTORY

Read before the Academy, Tuesday, April 23, 1935

Beginning in 1900 I have directed a continuous research extending over thirty-four years upon the gross and detailed evolution of two widely contrasting groups of quadrupeds and for the first time in the history of biology have succeeded in constructing two actual ancestral trees, as follows:

Titanothere tree, 15 branches of phyla, Lower Eocene to Lower Oligocene time inclusive—20,000,000 years (see Titanothere Monograph, 1929).

Proboscidea tree, 31 branches of phyla, Upper Eocene to Recent time, inclusive—50,000,000 years (see forthcoming Proboscidea Monograph, 1935–1936).

Titanotheres	*Proboscideans*
1. Defense by bony horns, arising as aristogenes at widely different and successive periods of geologic time.	1. Defense by superior tusks, effective against all carnivorous enemies.
2. Grinding teeth stationary in evolution, adaptive to a single browsing habit, evolution arrested. Aristogenes extremely limited. Extinction sudden due to world change of flora of the north temperate hemisphere.	2. Grinding teeth plastic, aristogenes numerous, with rapid radiation into 31 independent lines of form, adapted to the highly diversified flora from the Equator to the arctic tundras. Aristogenes extremely numerous and varied. Extinction of mastodonts gradual, due to progressive desiccation of the world flora. Extinction of elephants relatively recent.
3. Inferior incisive tusks degenerate and ineffective, disappearing.	3. Second pair of inferior incisors in mastodonts radiating adaptively into 31 different functions.
4. Migration limited to a single parallel in north latitude from North America westward only to the Balkans of Eurasia.	4. Migration from Africa through all the continents and climates of the globe except Australia. Migration apparently enforced and hastened by over-population.
5. Body and limb growth unspecialized, attaining gigantism.	5. Body and limbs unspecialized, attaining extreme gigantism.

These contrasting adaptive radiations in the proportions of the teeth, body and limbs and in the aristogenes of the grinding teeth afford a solution of the great problem of the *Modes of origin of species* since, among mammals, from the time of Linnæus to the present, species are chiefly defined by the biomechanical characters of the teeth and of the skeleton.

Not only the origin of species is revealed, but also the origin of all the lower to higher taxonomic divisions of classification: ascending mutations, subspecies, species, collective species, subgenera, genera, subfamilies, families, suborders and orders.

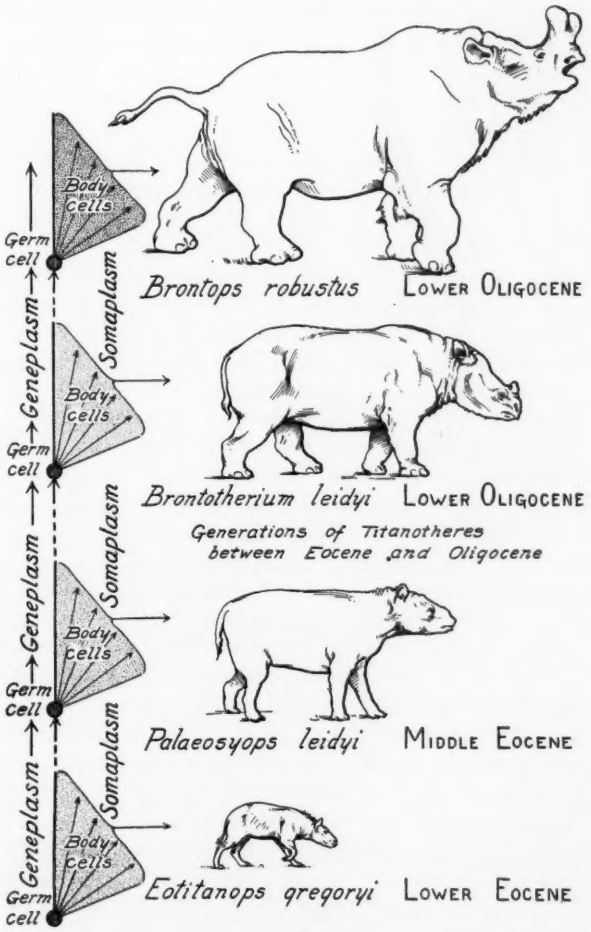

FIGURE 1

Continuous geneplasmic evolution of the Titanotheres during a 20,000,000 year period: Lower Eocene to Lower Oligocene. After Titanothere Monograph, Volume II, page 808, figure 727.

Equally significant and novel is the discovery that there are two profoundly different evolutionary modes in the origin of species:

1. The older modes known to naturalists from the time of Aristotle to that of Darwin, namely, changes of proportion or of degree which we term *ALLOIOMETRONS;* alloiometrons are governed by the action of the four well-known energetic factors, subject to Natural Selection.

2. Creative changes of kind, the origin of absolutely new characters, which we term *ARISTOGENES*, new adaptations arising directly from the geneplasm, e.g., the horns of Titanotheres and the innumerable new cones, crests and other elements in the teeth of Proboscideans.

Proboscidean aristogenes originate in the four cones of *Mœritherium;* all Proboscideans spring from four-coned ancestral grinders. *Phiomia osborni* with ten aristogenes evolves into *Amebelodon fricki* with an estimated twenty-five aristogenes, or the addition of approximately one aristogene or new adaptation every million years.

Principles of Geneplasmic Evolution.—Palæontology is greatly indebted to Weismann for establishing the principle of the continuity of the geneplasm. We record the details of the adaptive radiation of Titanotheres into fifteen branches of phyla in each of which proportional alloiometric evolution is responsible for changes of environment or habit. The outstanding biological discovery in the Titanotheres is the potentiality of horn origin in the geneplasm so that the horns independently appear at widely separated intervals of geologic time.

The five figures selected from the Titanothere and Proboscidea Monographs for this contribution are to be viewed as *projections into visible form of the potentialities of the geneplasm*, for example, in figure 1 the geneplasm of *Eotitanops gregoryi*, during a period of 20,000,000 years evolves into the geneplasm of *Brontops robustus*. In figure 4 during a period of 3,000,000 years the geneplasm of *Archidiskodon proplanifrons* of South Africa evolves into the geneplasm of *Archidiskodon maibeni* of Nebraska. While organisms like *Drosophila* reveal the mechanism, interrelations and, to a certain extent, functions and mutations of the genes, these recently discovered mammalian phyla, extending over enormous periods of time, reveal the evolution and adaptive radiation of the geneplasm with a clearness and fullness of detail never known to biologists before.

In figure 2 is shown the tremendous contrast between Darwin's knowledge of the actual modes of evolution when he formulated his theory of natural selection in the year 1859 and our present knowledge of the actual modes of evolution as now observed in the Proboscidea. With all contemporary naturalists he included all the Proboscidea within three genera—*Deinotherium, Elephas* and *Mastodon* and within the two last genera he knew of only ten species. Since the phylogeny of July, 1934, we now (April, 1935) know of thirty-one genera, two hundred twenty-nine species, sixteen subfamilies, seven families and two new suborders, *MŒRI-*

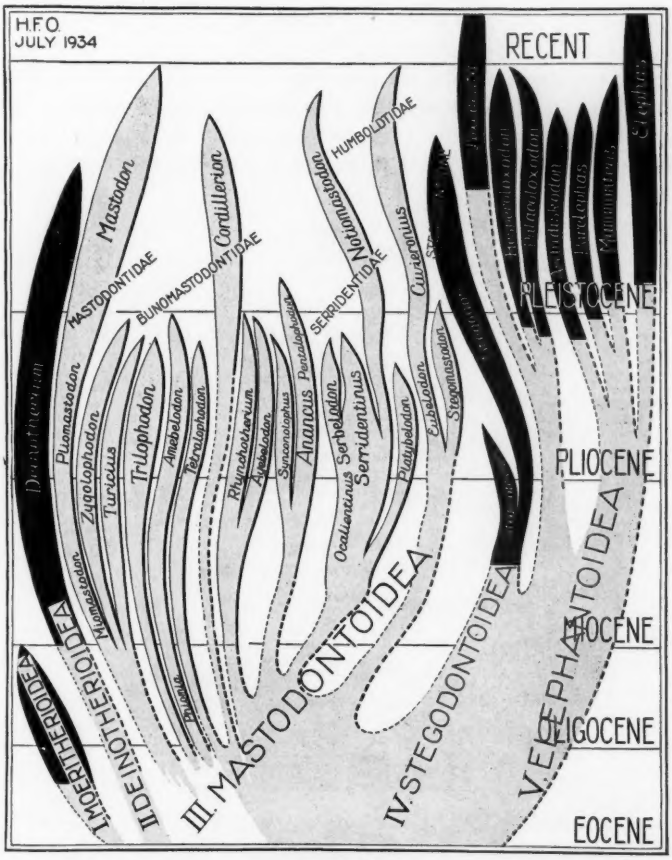

FIGURE 2

Geneplasmic radiation of the Proboscidea into five great orders (I–V) and twenty-eight lines of generic descent, so far as known to July, 1934. Proboscidea Monograph, Volume I.

Compare figures 3 (*Phiomia-Amebelodon*), 4 and 5 (*Archidiskodon*).

The earliest known Proboscideans are the aquatic MŒRITHERIOIDEA (I) of North Africa which imitate the hippopotami in dental and bodily form. Most aberrant are the DEINOTHERIOIDEA (II) of Africa and Eurasia with down-turned inferior tusks and tall elephantoid bodies. In the MASTODONTOIDEA (III) the single genus *Mastodon*, as known to Darwin in 1859, breaks up into four families, eleven subfamilies and twenty-three widely distinct genera shown in the central branches of the above diagram; each of these branches of the single Cuvier-Darwin genus *Mastodon* is sharply distinguished from others by the adaptations of the grinding teeth and inferior pair of incisive tusks. Most remarkable is the evolution of the shovel-tuskers (*Amebelodon*) as shown in figure 3.

The single genus *Elephas* of Cuvier-Darwin breaks up into two suborders (STEGODONTOIDEA, IV, and ELEPHANTOIDEA, V), four subfamilies and nine generic lines of descent.

THERIOIDEA and *STEGODONTOIDEA*, even excluding all the true Elephants or *ELEPHANTOIDEA*.

One of the most remarkable branches of the *MASTODONTOIDEA* is that of the shovel-tuskers of the Fayûm Oligocene of northern Egypt (genus *Phiomia*) which, after a tremendous journey and passage through a number of intermediate stages, reappears in the shovel-tusker discovered

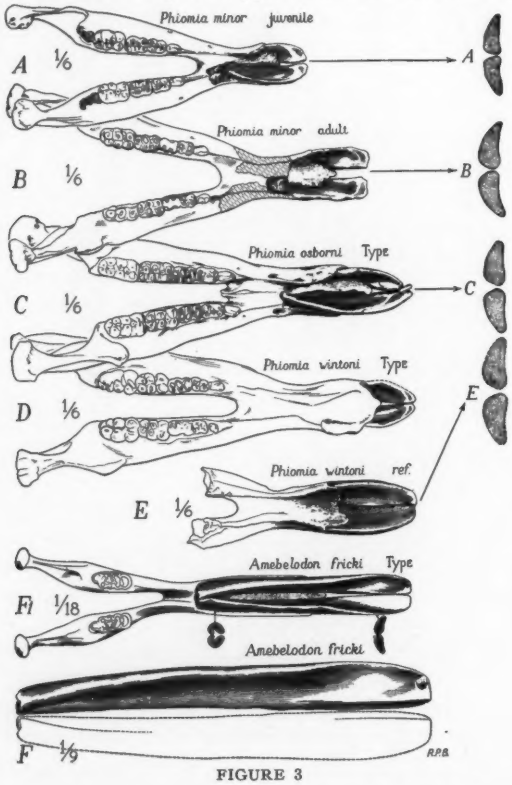

FIGURE 3

Geneplasmic evolution of the single mastodont Phylum Amebelodontinæ, the shovel-tuskers.

Thirty million year evolution of the lower jaw and paired inferior tusks from the *Phiomia minor* of the Oligocene of North Africa into the *Amebelodon fricki* of the Pliocene of Nebraska. It may be observed that the giant shovel-tusks of *Amebelodon fricki* dominate the entire evolution of the lower jaw and grinding teeth, of the skull, also of the skeleton. This is the most remarkable single phylum ever discovered up to the year 1934. See figure 4, *Archidiskodon*, as discovered up to April, 1935.

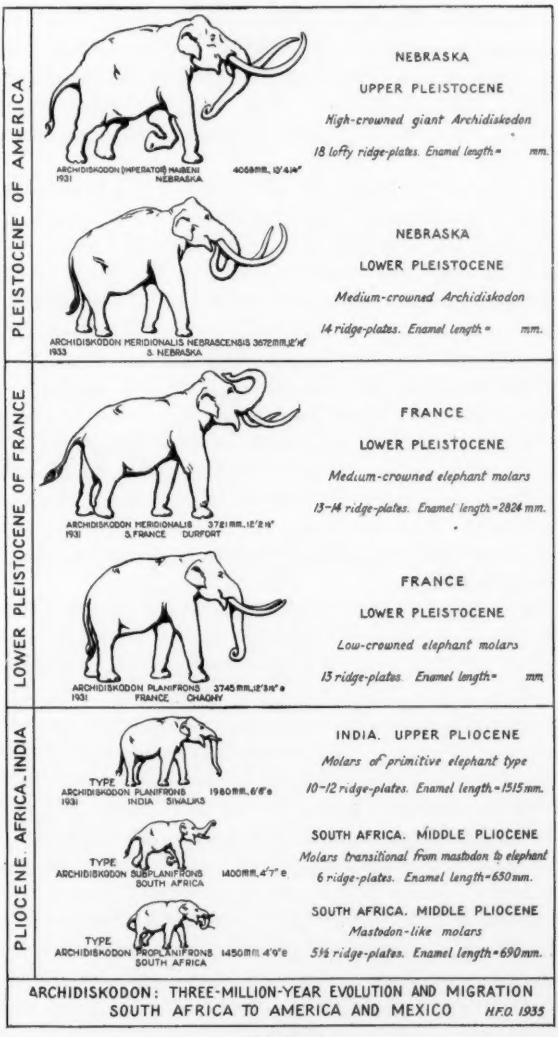

FIGURE 4

Geneplasmic evolution of the archaic-toothed mammoths during a three million year period so far as known to April, 1935.

Twenty-seven years of continuous exploration and research have yielded all the ascending stages in the geneplasm of this archaic-toothed mammoth. Compare figure 2 (*Archidiskodon*) and figure 5 (migration).

by E. H. Barbour in Nebraska (genus *Amebelodon*). These two shovel-tuskers, *Phiomia osborni* of North Africa and *Amebelodon fricki* of Nebraska, according to Drs. Merrill and Chaney, probably uprooted similar aquatic plants distributed along their migration route from northern Africa to North America; the entire anatomy is subject to the increasing dominance of the shovel-tusks. See figure 3.

In the *ELEPHANTOIDEA* we have discovered a brilliant example of aristogenic growth and extensive migration in the evolution of *Archidiskadon proplanifrons*, a very primitive stage found in the Vaal River gravels of South Africa, into the *Archidiskodon maibeni* of Nebraska. *Archidiskodon proplanifrons* has a molar crown pattern like that of a mastodont, with an enamel length of 690 mm.; *Archidiskodon maibeni* has eighteen tall ridge plates and an estimated enamel length of 9000 mm. This is the first time that the evolution of the elephantoid molar from a theoretic mastodont prototype has ever been actually demonstrated.

We owe these superb South African materials to the generosity and large-mindedness of the McGregor Museum in Kimberley, South Africa, under Director Wilman, an instance of coöperation of all the museums and of all the palaeontologists of the world in a research which has occupied twenty-seven years, from 1907, the author's expedition to the Fayûm of northern Egypt to the present time.

The 15,000 mile migration of *Archidiskodon*, from the Vaal River of South Africa through intermediate stages in North Africa, France, Italy, Britain, India, then the long geographic break to the Niobrara River in Nebraska where Leidy discovered these animals in 1858, is the most remarkable trek of any species of plant or animal thus far discovered in the life history of the earth. The by-product is the fact that these stages give us an unprecedented opportunity of measuring precisely the actual rate of evolution between Upper Pliocene and Middle Pleistocene time. The enamel length increasing from 690 mm. to an estimated 9000 mm. affords an evolution of approximately 15 mm. of enamel length per thousand years.

Weismann and Mendel have inspired the marvelous discoveries in the mechanism of heredity during the past fifty-four years since Weismann's enunciation of the principle of the continuity of the geneplasm. In the opinion of the present contributor attempts by De Vries and the distinguished successors of Bateson in the Mendelian school of thought fail to establish the mutational origin of a single species so far as species are defined in their biomechanical characters.

So far as biomechanics bear upon the problem, current hypotheses as to the sudden or mutational origin of species entertained by T. H. Morgan and other leaders of the experimental and genetic schools are absolutely negatived by the actual modes of the origin of species discovered during

these thirty-four years of research. A new aristogene is readily distinguished from a new D. mutation by invariably obeying the eighteen principles of biomechanical adaptation; D. mutations on the contrary may or may not be adaptive.

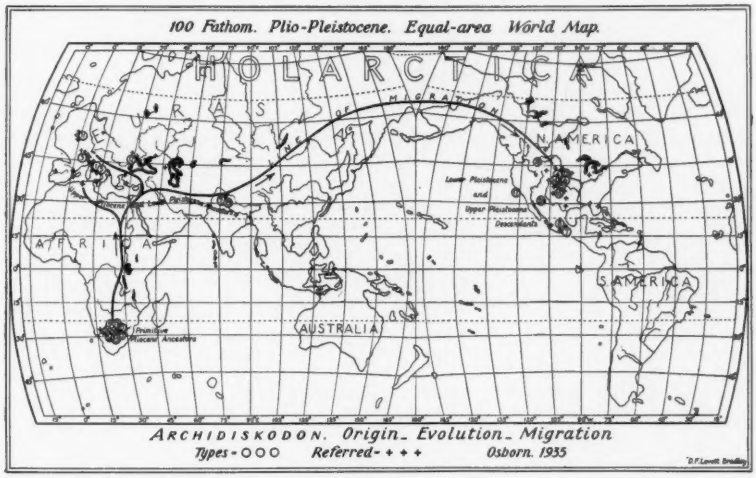

FIGURE 5

Successive habitats and world migration routes of the archaic-toothed mammoth *Archidiskodon*. Sterling World Map on the 600 Fathom Line.

Compare figures 2 and 4.

VAAL RIVER GRAVELS OF SOUTH AFRICA, habitat of the recently discovered primitive Mio-Pliocene ancestors of *Archidiskodon*.

SIWALIK HILLS OF NORTHERN INDIA, habitat of the Upper Pliocene-Lower Pleistocene successors (*Archidiskodon planifrons*) described by Falconer and Cautley in the year 1846 (1845).

SOUTHWESTERN EURASIA, habitat of the Archidiskodonts intermediate between *Archidiskodon planifrons* of northern India, and the southern mammoth *Archidiskodon meridionalis* discovered in Italy and described by Nesti in the years 1808, 1825.

(During this era of Upper Pliocene and Lower Pleistocene time, *Archidiskodon* disappeared in western and southern Eurasia and migrated to the hospitable plains of North America.)

NEBRASKA TO MEXICO. Lower and Upper Pleistocene descendants of the *Archidiskodon meridionalis* of southern France (compare figure 4), evolving to the close of Third Interglacial time in the Pleistocene epoch into the gigantic *Archidiskodon maibeni* of Nebraska.

On the contrary the actual modes of the origin of species and of all the other steps in the origin and divergence of biomechanical characters are now thoroughly established by consistent discoveries dating back to those of Waagen in 1869, and culminating in the discovery of eighteen principles

of origin revealed in the widely contrasting evolution of the Titanotheres and of the Proboscideans.

[1] This is the thirteenth contribution by the author on the "Origin of Species" and the principles of biomechanical evolution, the series beginning in the year 1925.

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